

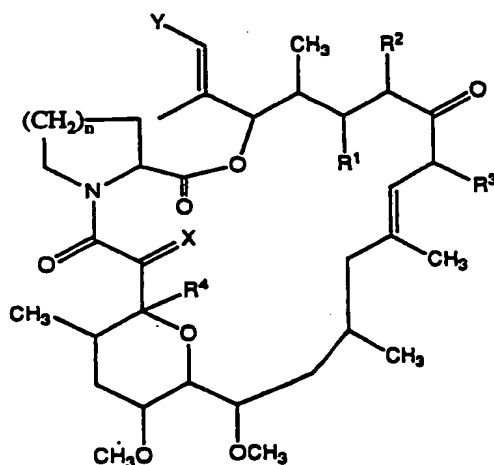


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(54) Title: IMMUNOSUPPRESSIVE MACROCYCLIC COMPOUNDS



(57) Abstract

There are provided compounds of formula (I), wherein R¹ represents H, OH or alkoxy; R² represents H; in addition, R¹ and R² may together represent a second bond between the carbon atoms to which they are attached; R³ represents methyl, ethyl, propyl or allyl; R⁴ represents H, OH, alkyl, alkoxy, halogen, amino, S-alkyl, NHCHO or NHCO-alkyl; n represents 1 or 2; X represents O, (H, OH), (H, H) or =NH; and Y represents an optionally substituted cyclohexyl or substituted cyclopentyl group; with various provisos. The compounds are useful, inter alia, as immunosuppressive agents.

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IMMUNOSUPPRESSIVE MACROCYCLIC COMPOUNDS

This invention relates to immunosuppressive macrocyclic compounds, processes for their preparation, their use as medicaments, and compositions containing them.

European Patent Application 184162 (to Fujisawa Pharmaceuticals Co Ltd) discloses a number of macrocyclic compounds isolated from microorganisms belonging to the genus Streptomyces. The macrolides are numbered FR-900506, FR-900520, FR-900523 and FR-900525, and the preparation of some of their derivatives is also described.

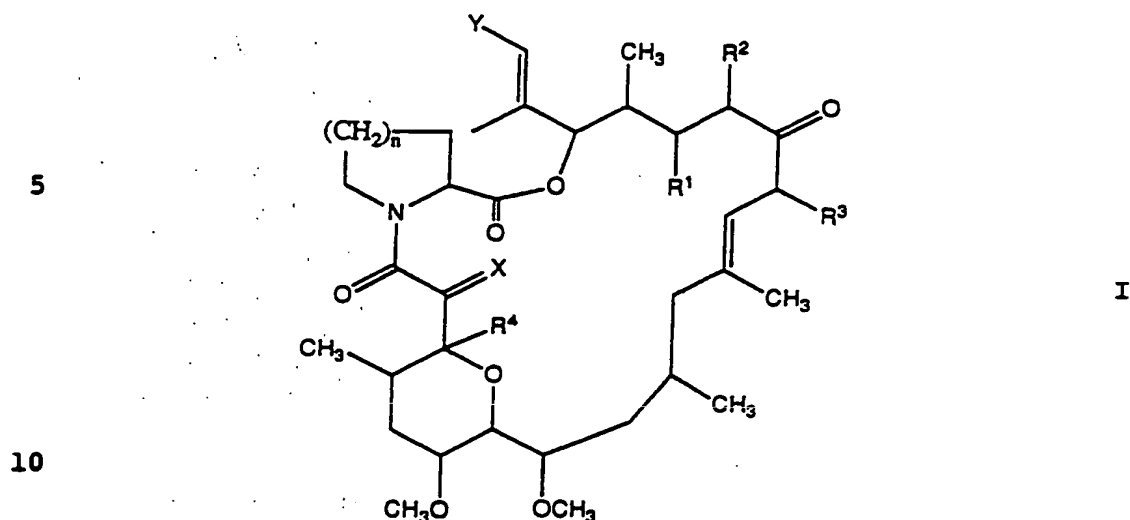
International Patent Applications Nos WO 89/05304 and PCT/GB90/01262 and European Patent Application No 413532 (to Fisons plc), European Patent Application 353678 (to Fujisawa Pharmaceuticals Co Ltd), European Patent Applications 349049, 349061, 358508 and 388153 (to Merck & Co Inc) and European Patent Application 356399 and International Patent Application WO 90/15805 (to Sandoz AG) also disclose a number of immunosuppressive macrocyclic compounds.

We have now found a new group of immunosuppressive macrocyclic compounds which possess advantageous properties over those disclosed previously.

According to the present invention, there is provided a

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compound of formula I,



wherein

R^1 represents H, OH or alkoxy;

15 R^2 represents H;

in addition, R^1 and R^2 may together represent a second bond between the carbon atoms to which they are attached;

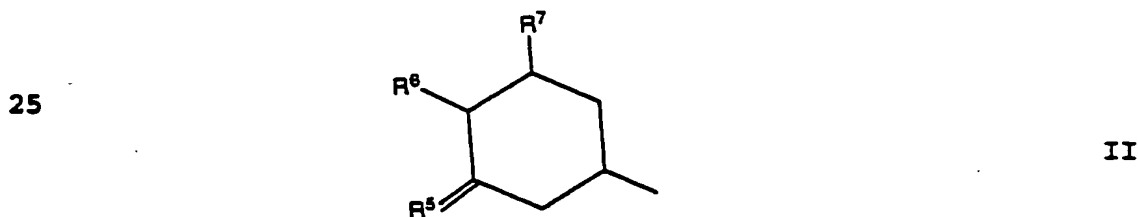
R^3 represents methyl, ethyl, propyl or allyl;

R^4 represents H, OH, alkyl, alkoxy, halogen, amino,
20 S-alkyl, NHCHO or NHCO-alkyl;

n represents 1 or 2;

X represents O, (H,OH), (H,H) or =NH; and

Y represents a cyclic group of formula II,



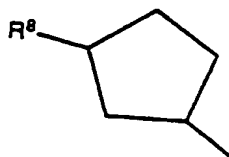
in which R^5 represents (H,H), (H,OH), (H, methoxy) or O;

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R^6 represents H, (R)-OH, (S)-OH, alkoxy, amino, alkylamino, alkanoylamino, formyloxy or halogen; R^7 represents H; and in addition R^5 and R^6 may together represent a second bond between the carbon atoms to which they are attached; or R^6 and R^7 may together represent a second bond between the carbon atoms to which they are attached;

~~or a cyclic group of formula III,~~

10



III

in which R^8 represents alkyl substituted by one or more groups selected from OH, alkoxy, =O, and CO_2H ; or alkenyl optionally substituted by one or more groups selected from OH, =O, or CO_2H ;

provided that

a) when n represents 1; R^1 represents OH; R^3 represents allyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent O;

b) when n represents 2;

i) R^1 represents OH; R^3 represents methyl, ethyl, allyl or propyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent O;

ii) when R^1 and R^2 together represent a second bond

- between the carbon atoms to which they are attached or each represent H; R^3 represents allyl or propyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent O;
- 5 iii) when R^1 represents OH, methoxy or together with R^2 it represents a second bond between the carbon atoms to which they are attached; R^3 represents allyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents methoxy; then X does not represent O;
- 10 iv) when R^1 represents H or OH; R^3 represents allyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent (H, OH);
- v) when R^1 represents H; R^3 represents propyl; R^4 represents OH; R^5 represents (H, OH); and R^6 represents
- 15 (R)-OH; then X does not represent O;
- vi) when R^1 represents OH; R^3 represents ethyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent (H, OH);
- vii) when R^1 and R^2 together represent a second bond
- 20 between the carbon atoms to which they are attached or each represent H; R^3 represents ethyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent O;
- viii) when R^1 represents OH; R^3 represents allyl; R^4
- 25 represents OH; R^5 represents (H, OH) or (H, methoxy); and R^6 represents (R)-OH; then X does not represent (H, H);
- ix) when R^1 represents OH; R^3 represents ethyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6

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- r presents (R)-OH; then X does not r present (H,H);
- x) when R^1 represents OH; R^3 represents methyl, ethyl or allyl; R^4 represents OH; R^5 represents (H,OH); and R^6 represents (R)-OH; then X does not represent O; and
- 5 xi) when R^1 represents OH; R^3 represents allyl; R^4 represents OH; R^5 represents O; and R^6 represents (R)-OH; then X does not represent O;

and pharmaceutically acceptable derivatives thereof.

- 10 Pharmaceutically acceptable derivatives which may be mentioned include esters, amides and salts of any carboxylic acid groups which may be present. The esters and amides preferably contain up to 6 carbon atoms. Salts include alkali-metal and alkaline earth metal salts, for
- 15 example sodium or calcium.

When any one of R^1 , R^4 , R^5 , R^6 , and R^8 represent carbon-containing groups, we prefer those groups to contain up to 10 carbon atoms, more preferably up to 6 carbon

20 atoms.

Groups which R^8 may represent include CHO and CO₂H.

Preferably, R^1 represents H or OH. We prefer R^4 to

25 represent H, OH, alkyl, halogen or amino. Desirably, R^5 represents (H,OH) or (H, methoxy). Preferably R^6 represents H, (R)-OH or amino. We prefer R^8 to represent an amide of a CO₂H group or alkyl substituted by alkoxy.

Subgroups of compounds which may be mentioned include: compounds of formula I in which Y represents a cyclic group of formula III; compounds of formula I in which R⁴ represents alkoxy; compounds of formula I in which R⁴ represents amino, alkylamino, alkanoylamino, halogen and thioalkyl; compounds of formula I in which R⁴ represents H or alkyl; and compounds of formula I in which R⁶ represents H, (S)-OH or halogen or together with R⁵ represents a second bond between the carbon atoms to which they are attached or together represent a pair of vicinal hydrogen atoms.

A preferred group of specific compounds which may be mentioned is:

- 15 17-allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 20 17-allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid morpholine amide)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 25 17-allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 17-allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-1,13,19,21,27-pentamethyl-

- 11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone;
- 17-allyl-1-amino-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclo
hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
5 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
ene-2,3,10,16-tetraone;
- 17-allyl-1-fluoro-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclo
hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
10 18-ene-2,3,10,16-tetraone;
- 17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol (methyl
ether))-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
ene-2,3,10,16-tetraone; and
- 15 17-Allyl-1,14-dihydroxy-12-[2-(4-amino-3-methoxycyclohexyl)-
1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone.

20 The compounds disclosed in the above-mentioned applications
may be used as starting materials for the production of
compounds of the present invention. Alternatively, they
may be prepared by total synthesis.

25 According to a further aspect of the invention, there is
provided a process for the production of a compound of
formula I as defined in claim 1, which comprises:

(a) producing a compound of formula I in which R¹ and

R^2 together represent a second carbon-carbon bond between the carbon atoms to which they are attached, by dehydration of a corresponding compound in which R^1 represents OH and R^2 represents H;

5 (b) producing a compound of formula I in which R^1 and R^2 each represent hydrogen, by reduction of a corresponding compound in which R^1 and R^2 together represent a second carbon-carbon bond between the carbon atoms to which they are attached;

10 (c) producing a compound of formula I in which X represents (H,OH), by reduction of a corresponding compound in which X represents O;

(d) producing a compound of formula I in which X represents (H,H), by reduction of a corresponding compound
15 in which X represents O;

(e) producing a compound of formula I in which X represents O, by oxidation of a corresponding compound in which X represents (H,OH);

(f) producing a compound of formula I in which R^4
20 represents alkoxy, by reaction of a corresponding compound in which R^4 represents OH and X represents (H,OH) with an alkanol;

(g) producing a compound of formula I in which R^4 represents halogen, by reaction of a corresponding compound
25 in which R^4 represents OH with a suitable halogenating agent;

(h) producing a compound of formula I in which R^4 represents H or alkyl, by reaction of a corresponding

compound in which R^4 represents halogen with an organometallic reagent;

(i) producing a compound of formula I in which R^4 represents amino, by reaction of a corresponding compound
5 in which R^4 represents halogen with ammonia;

(j) producing a compound of formula I in which X represents =NH, by reaction of a corresponding compound in
which X represents O with ammonia;

(k) producing a compound of formula I in which R^4
10 represents S-alkyl, by reaction of a corresponding compound in which R^4 represents halogen with an alkylthiol;

(l) producing a compound of formula I in which R^4 represents NHCHO, by reaction of a corresponding compound in which R^4 represents amino with formic acid;

15 (m) producing a compound of formula I in which R^4 represents NHCO-alkyl, by reaction of a corresponding compound in which R^4 represents amino with an alkanoic anhydride;

(n) producing a compound of formula I in which R^6
20 represents (S)-OH, by elimination of a leaving group from a corresponding compound in which R^6 represents the leaving group;

(o) producing a compound of formula I in which R^6 represents H and R^5 represents O, by elimination of a
25 leaving group from a corresponding compound in which R^6 represents the leaving group;

(p) producing a compound of formula I in which R^6 and R^7 together represent a second bond between the carbon

atoms to which they are attached, by elimination of a leaving group from a corresponding compound in which R^6 represents the leaving group;

(q) producing a compound of formula I in which Y represents a cyclic group of formula III and R^8 represents CHO, by elimination of a leaving group from a corresponding compound in which R^6 represents the leaving group;

(r) producing a compound of formula I in which R^6 represents halogen, by reaction of a corresponding compound in which R^6 represents a leaving group with halide ion;

(s) producing a compound of formula I in which R^5 and R^6 together represent a second bond between the carbon atoms to which they are attached, by elimination of halogen and alkoxy from a corresponding compound in which R^5 represents alkoxy and R^6 represents halogen;

(t) producing a compound of formula I in which R^5 represents (H,H) and R^6 represents H, by reduction of a corresponding compound in which R^5 and R^6 together represent a second bond between the carbon atoms to which they are attached;

(u) producing a compound of formula I in which R^6 represents H, by the action of hydride on a corresponding compound in which R^6 represents a leaving group;

(v) producing a compound of formula I in which R^6 represents amino, by reduction of a corresponding compound in which R^6 represents azido;

(w) producing a compound of formula I in which R^6

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represents alkylamino or alkanoylamino, by reaction of a corresponding compound in which R^6 represents amino with a suitable alkylating or acylating reagent;

(x) producing a compound of formula I in which R^8 represents alkyl substituted by OH, by reduction of a corresponding compound in which R^8 represents alkyl substituted by =O;

(y) producing a compound of formula I in which R^8 includes a carboxylic acid group, by oxidation of a corresponding compound in which R^8 includes an aldehyde group; and

(z) producing a compound of formula I in which R^8 represents optionally substituted alkenyl, by a Wittig reaction between a corresponding compound in which R^8 includes an aldehyde and an appropriate Wittig reagent.

In process (a), the dehydration may be carried out in a solvent which does not adversely affect the reaction (eg toluene), in the presence of a trace amount of acid (eg p-toluenesulphonic acid), at a temperature of from 50 to 100°C.

In processes (b) and (t), the reduction may be carried out catalytically using hydrogen. Suitable catalysts include platinum catalysts (eg platinum black, platinum oxides), palladium catalysts (eg palladium oxides, palladium on charcoal), nickel catalysts (eg nickel oxide, Raney Nickel), and rhodium catalysts (eg rhodium on alumina).

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Suitable solvents are those which do not adversely affect the reaction, and include methanol, ethanol, ethyl acetate, dichloromethane and dimethylformamide. The reduction may be carried out at or around room temperature.

5

In process (c), suitable reagents for the reduction include tri-ⁿbutyltin hydride in a solvent which does not adversely affect the reaction (eg toluene) at a temperature of from 50 to 100°C, sodium borohydride, zinc in acetic
10 acid at or around room temperature, sodium triacetoxyborohydride in acetic acid, L-Selectride
(Registered Trade Mark) in tetrahydrofuran, or borane/^tbutylamine complex in a solvent such as methanol or ethanol.

15

In process (d), the reduction may be achieved by the action of H₂S, preferably in the presence of pyridine or an amine (for example morpholine), in a solvent which does not adversely affect the reaction (for example
20 dimethylformamide, pyridine or methanol), at or around room temperature.

In process (e), the oxidation may be carried out in the presence of a suitable oxidizing agent, such as cupric
25 acetate. Suitable solvents include those which do not adversely affect the reaction, for example methanol. The reaction may be carried out up to the reflux temperature of the solvent.

In process (f), the reaction may be carried out in the presence of a suitable acid catalyst, for example montmorillonite K10. The solvent used may conveniently be the alkanol reagent, and the reaction may be carried out at or around room temperature.

~~In process (g), suitable halogenating agents include diethylaminosulphur trifluoride and thionyl chloride.~~ The halogenation is preferably carried out in a solvent which does not adversely affect the reaction, for example dichloromethane, at or below room temperature, and preferably under an inert atmosphere.

15 In process (h), suitable organometallic reagents include lithium dialkyl copper reagents, which may be prepared from a copper halide and an alkyl lithium reagent. R^4 preferably represents Cl in the starting material. Suitable solvents include those which do not adversely affect the reaction, for example diethyl ether. The reaction is preferably carried out at reduced temperature.

In processes (i) and (j), suitable solvents include those which do not adversely affect the reaction, for example 25 diethyl ether. R^4 preferably represents Cl in the starting material. The reaction may be carried out at or around room temperature.

In process (k), suitable solvents include those which do not adversely affect the reaction, for example tetrahydrofuran (THF). R^4 preferably represents Cl in the starting material. The reaction may be carried out at
5 or around room temperature.

In process (l), the solvent is conveniently formic acid. The reaction may be carried out at or around room temperature, and in the presence of acetic anhydride.

10

In process (m), suitable solvents include those which do not adversely affect the reaction, for example methanol. The reaction may be carried out at below room temperature.

15 In processes (n)-(q), suitable leaving groups include tosylate, mesylate and triflate (trifluoromethylsulphonyloxy), and the elimination is carried out in the presence of an acid catalyst, preferably silica. The leaving group may be introduced by reaction of
20 a compound of formula I in which R^6 represents (R)-OH with a suitable reagent, for example trifluoromethanesulphonic acid anhydride.

In process (r), suitable leaving groups include tosylate,
25 mesylate and triflate. Suitable sources of halide include tetra- n -butylammonium halides, for example tetra- n -butylammonium iodide. Suitable solvents include those which do not adversely affect the reaction, for

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example benzene. The reaction may be carried out at or around room temperature.

In process (s), the elimination is preferably carried out by the action of powdered zinc. The solvent is preferably acetic acid and the reaction may be carried out at or around room temperature.

In process (u), suitable leaving groups include imidazol-1-yl(thiocarbonyl)oxy, which may be introduced by reaction of a corresponding compound in which R^6 represents OH with 1,1'-thiocarbonyldiimidazole. Suitable sources of hydride include tributyltin hydride, and the reaction is preferably carried out in the presence of AIBN. Suitable solvents include those which do not adversely affect the reaction, for example benzene. The reaction may be carried out up to the reflux temperature of the solvent.

In process (v), suitable reducing agents include 1,3-propanedithiol. Suitable solvents include those which do not adversely affect the reaction, for example methanol. The reaction is preferably carried out in the presence of triethylamine, and may be carried out at or around room temperature. The azido compound may be produced by the action of azide ion on a corresponding compound in which R^6 represents a leaving group, for example triflate.

In process (w), suitable alkylating agents include methyl iodide, and suitable acylating agents include acyl halides, for example acetyl chloride. Suitable solvents include
5 those which do not adversely affect the reaction, for example dichloromethane. The reaction may be carried out at or around room temperature.

In process (x), suitable reducing agents include
10 L-Selectride. Suitable solvents include those which do not adversely affect the reaction, for example THF. The
~~reaction is preferably carried out below room temperature.~~

In process (y), suitable oxidizing agents include sodium
15 chlorite, preferably in the presence of 1-methylcyclohex-1-ene. Suitable solvents include those which do not adversely affect the reaction, for example
tbutanol. The reaction is preferably carried out at or around room temperature.

20

In process (z), suitable Wittig reagents include
~~=(carbomethoxymethylene)triphenylphosphorane.~~ Suitable
solvents include those which do not adversely affect the
reaction, for example toluene. The reaction may be carried
25 out at or around the reflux temperature of the solvent.
Conventional methods may then be used to produce the
corresponding acid and amides from the product obtained
with this preferred reagent.

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Where necessary, hydroxy groups in intermediate compounds may be protected using conventional protecting group chemistry [as described in "Protective Groups in Organic Chemistry", ed: J W F McOmie, Plenum Press (1973), and "Protective Groups in Organic Synthesis", T W Greene, Wiley-Interscience (1981)]. A particularly useful protecting group which may be mentioned is tbutyldimethylsilyl.

10

Compounds in which R^4 represents halogen and compounds in which R^6 represents a leaving group are useful in the production of corresponding compounds of formula I.

15 The compounds of formula I may be isolated from their reaction mixtures using conventional techniques.

The compounds of formula I are useful because they possess pharmacological activity in animals; in particular they are useful because they possess immunosuppressive activity, eg
20 in the tests set out in Tests A, B, C and D. Thus the compounds are indicated for use in the treatment or prevention of resistance to transplanted organs or tissues, such as kidney, heart, lung, bone marrow, skin, cornea, etc; and of autoimmune, inflammatory, proliferative and
25 hyperproliferative diseases, and of cutaneous manifestations of immunologically-mediated diseases: for example rheumatoid arthritis, lupus erythematosus, systemic

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lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, nephrotic syndrome, psoriasis, atypical dermatitis, contact dermatitis and further eczematous dermatitides, seborrheic
5 dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Alopecia areata, eosinophilic fasciitis, atherosclerosis etc.

10 The compounds of the invention are also indicated more generally in the treatment of respiratory diseases, for example reversible obstructive airways disease.

Further, the compounds of the invention are indicated in
15 the treatment of a disease selected from intestinal inflammations/allergies such as Coeliac disease, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis; and food related allergic diseases which have symptomatic manifestation remote from the
20 gastro-intestinal tract, for example migraine, rhinitis and eczema.

The compounds of the invention are also indicated for use as antimicrobial agents, and thus may be used in the treatment
25 of diseases caused by pathogenic microorganisms and the like.

We therefore provide the use of compounds of formula I as

pharmaceuticals.

Further, we provide the use of a compound of formula I in the manufacture of a medicament for use as an immunosuppressive agent.

For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired (eg topical, parenteral or oral) and the disease indicated. However, in general, satisfactory results are obtained when the compounds are administered at a daily dosage of from 0.001 to 20mg per kg of animal body weight.

For man the indicated total daily dosage is in the range of from 0.01mg to 1000mg and preferably from 0.5mg to 100mg, which may be administered, for example twice weekly, or in divided doses from 1 to 6 times a day or in sustained release form. Thus unit dosage forms suitable for administration, eg oesophageally, comprise from 0.01mg to 500mg, and preferably 0.5mg to 100mg of the compound preferably admixed with a solid or liquid pharmaceutically acceptable diluent, carrier or adjuvant.

According to our invention we also provide a pharmaceutical composition comprising preferably less than 80%, and more preferably less than 50% by weight, of a compound of formula I in combination with a pharmaceutically acceptable

adjuvant, diluent or carrier. Examples of suitable adjuvants, diluents or carriers are: for tablets, capsules and dragees - microcrystalline cellulose, calcium phosphate, diatomaceous earth, a sugar such as lactose, dextrose or mannitol, talc, stearic acid, starch, sodium bicarbonate and/or gelatin; for suppositories - natural or hardened oils or waxes; and for inhalation compositions - coarse lactose. The compound of formula I preferably is in a form having a mass median diameter of from 0.01 to 10 μ m. The compositions may also contain suitable preserving, stabilising and wetting agents, solubilisers (eg a water-soluble cellulose polymer such as hydroxypropyl methylcellulose, or a water-soluble glycol such as propylene glycol), sweetening and colouring agents and flavourings. The compositions may, if desired, be formulated in sustained release form.

For the treatment of reversible obstructive airways disease, we prefer the compound of formula I to be administered by inhalation to the lung, especially in the form of a powder.

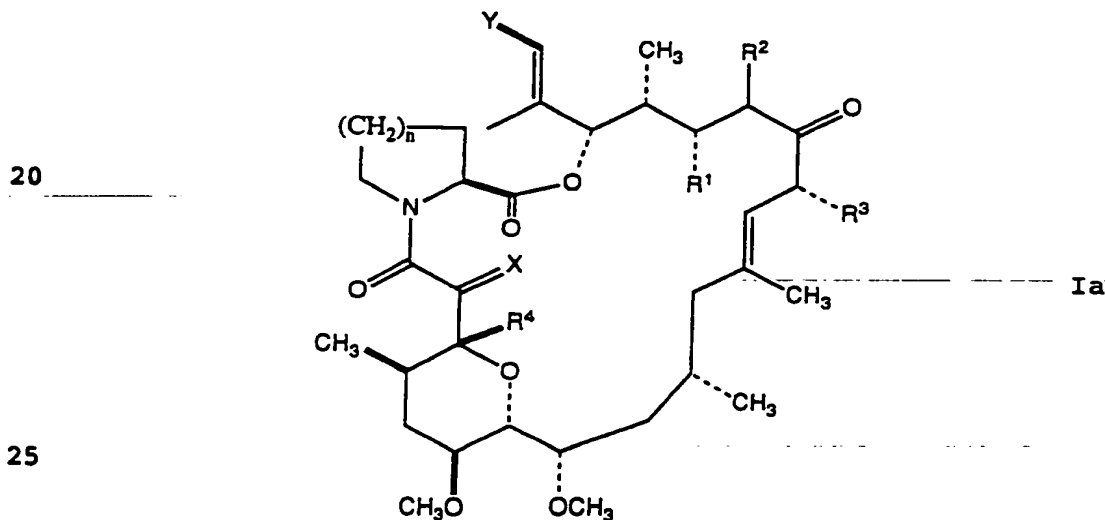
According to a further aspect of the invention, there is provided a method of effecting immunosuppression which comprises administering a therapeutically effective amount of a compound of formula I, as defined above, to a patient.

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The compounds of formula I have the advantage that they are less toxic, more efficacious, are longer acting, have a broader range of activity, are more potent, are more stable, produce fewer side effects, are more easily absorbed or have other useful pharmacological properties, than compounds previously used in the therapeutic fields mentioned above.

The compounds of formula I have a number of chiral centres and may exist in a variety of stereoisomers. The invention provides all optical and stereoisomers, as well as racemic mixtures. The isomers may be resolved or separated by conventional techniques.

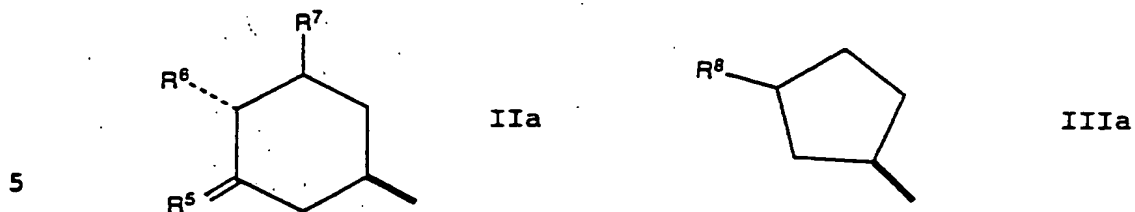
However, the preferred stereochemistry of various chiral carbon atoms are shown in formula Ia,



wherein R^1 to R^4 , X and n are as first defined above,

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and Y represents a cyclic group of formula IIa or IIIa,



in which R⁵ to R⁸ are as first defined above.

Test A

Mixed Lymphocyte Reaction (MLR) I

- 10 The MLR test was performed in microtitre plates, with each well containing 5×10^5 C57BL/6 responder cells (H-2^b), 5×10^5 mitomycin C treated (25 μ g/ml mitomycin C at 37°C for 30 minutes and washed three times with RPMI 1640 medium) BALB/C stimulator cells (H-2^d) in 0.2ml RPMI 1640
- 15 medium supplemented with 10% fetal calf serum, 2mM sodium hydrogen carbonate, penicillin (50 μ g/ml) and streptomycin (50 μ g/ml). The cells were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% of air for 68 hours and pulsed with ³H-thymidine
- 20 (0.5 μ Ci) 4 hours before the cells were collected. The object compound of this invention was dissolved in ethanol and further diluted in RPMI 1640 medium and added to the cultures to give final concentrations of 0.1 μ g/ml or less.

25 Test B

Mixed Lymphocyte Reaction (MLR) II

The MLR test was performed in 96-well microtitre plates with each well containing 3×10^5 cells from each of two

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responding donors in a final volume of 0.2ml RPMI 1640 medium supplemented with 10% human serum, L-glutamine and penicillin/streptomycin. The compound under test was dissolved at 10mg/ml in ethanol and further diluted in RPMI 1640. The cells were incubated at 37°C in a humidified atmosphere at 5% carbon dioxide for 96 hours. 3H-thymidine (0.5µCi) was added for the final 24 hours of the incubation to provide a measure of proliferation.

Test C

10 Graft versus Host Assay (GVH)

Spleen cells from DA and DAXLewis F1 hybrid rats were prepared at approximately 10^8 cells/ml. 0.1ml of these suspensions were injected into the rear footpads of DAXLewis F1 rats (left and right respectively). Recipient animals are dosed with the compound under test, either orally or subcutaneously, on days 0-4. The assay is terminated on day 7 when the popliteal lymph nodes of the animals are removed and weighed. The increase in weight of the left node relative to the weight of the right is a measure of the GVH response.

Test D

Inhibition of Interleukin-2 (IL-2) secretion

The test was performed following the method of S Sawada et al, J Immunol (6), Vol 139, pp1797-1803, but using the Jurkat cell line.

The invention is illustrated, but in no way limited, by the following Examples.

Example 1

17-Allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-1,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-

5 2,3,10,16-tetraone

(a) 17-Allyl-2,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-1,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

10 17-Allyl-1,2,14-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione (the compound of Example 5, WO 89/05304) (200mg) was added to a suspension of
15 montmorillonite K10 (500mg) in methanol (5ml). After stirring for 4 days at room temperature a further portion of montmorillonite was added (500mg) and stirring was continued for a further 2 days. The reaction mixture was then filtered through celite and was concentrated to an oil
20 in vacuo. Column chromatography on silica then gave the subtitle compound as an oil (42mg).

MS: 843 [M+Na]⁺; 904 [M+Rb]⁺

(b) 17-Allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-1,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
25 18-ene-2,3,10,16-tetraone

The compound of step (a) (40mg) was dissolved in methanol (3ml) and to this was added cupric acetate (100mg). The

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resulting suspension was stirred and heated to reflux for 30 minutes. The reaction mixture was then cooled, filtered and evaporated in vacuo. Column chromatography on silica gave the title compound (30mg) as an oil.

5 MS (FAB): 902.5 [M+Rb]⁺; 840.8 [M+Na]⁺; 818.8 [M+H]⁺; 800.8 [M+H]⁺; 786.8 [M+H-CH₃OH]⁺

¹³C NMR δ: 211.7 (C16); 197.6 (C2); 169.3 (C10); 166.2 (C3); 139.1 (C29); 130.5 (C31); 123.4 (C18); 116.7 (C42); 102.4 (C1); 102.4 (C1); 50.6 (C1-OCH₃)

10 Example 2

17-Allyl-1-amino-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

15 and

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-2-imino-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

20 (a) 17-Allyl-1-chloro-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (FR-900506) (0.6g) in dry dichloromethane (15ml) was added

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dropwise over 5 minutes at room temperature under an atmosphere of nitrogen to a solution of thionyl chloride (544 μ l) and pyridine (1.33ml) in dry dichloromethane (15ml). After stirring for 5 minutes at room temperature the reaction mixture was added slowly to vigorously stirred saturated aqueous sodium hydrogen carbonate solution (50ml). After stirring for 5 minutes this mixture was extracted with diethyl ether (150ml) and the extract washed with dilute aqueous hydrochloric acid (1M, 50ml), water and brine before being dried (MgSO₄), filtered and evaporated in vacuo to give the subtitle compound as a foam (630mg).

MS: 908.4 [M+Rb]⁺; 906.4 [M+Rb]⁺; 870.7 [M-HCl+Rb]⁺; 844.9 [M+H]⁺

¹³C NMR (CDCl₃) δ : 212.1 (C16); 189.3 (C2); 169.3 (C10); 164.1 (C3); 140.4 (C19); 135.8 (C41); 132.3 (C29); 129.4 (C31); 122.6 (C18); 116.6 (C42); 108.9 (C1); 84.3 (C34); 70.3 (C14); 48.2 (C20); 41.3 (C13); 9.8 (C39)

(b) 17-Allyl-1-amino-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

and

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-2-imino-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

A crude sample of the compound of step (a) (405mg) was taken up in THF (tetrahydrofuran) (8ml) and to this was

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added concentrated aqueous ammonia solution (4ml). After stirring for 20 minutes at room temperature the reaction mixture was diluted with water (20ml) and diethyl ether (50ml). The organic extract was then separated and washed with brine before being dried (MgSO_4), filtered and concentrated in vacuo to a foam. This was chromatographed on silica using HPLC eluting with 2% methanol in diethyl ether to give fraction A (190mg) and fraction B (98mg). Fraction A was further purified by chromatography on silica using HPLC eluting with ethyl acetate to give the first title compound (92mg) as a foam.

MS: 887.5 $[\text{M}+\text{Rb}]^+$; 803.7 $[\text{M}+\text{H}]^+$

^{13}C -NMR (CDCl_3) δ : 213 (C16); 198.2 (C2); 169.2 (C10); 166.2 (C3); 139.4 (C19); 135.7 (C41); 132.6 (C29); 129.6 (C31); 122.2 (C18); 116.5 (C42); 88.6 (C1); 84.2 (C34); 76.7 (C12); 75.5 (C23); 71.1 (C24); 70.2 (C14); 56.4 (C9); 52.7 (C17); 48.6 (C20); 43.0 (C15); 39.9 (C13); 38.9 (C5); 31.3 (C36); 30.7 (C37); 27.9 (C8); 26.1 (C21); 24.6 (C6); 21.3 (C7); 20.4 (C44); 14.2 (C30); 9.5 (C39)

Fraction B was further purified by chromatography on silica using HPLC eluting with hexane/acetone [2:1] to give the second title compound (70mg) as a foam.

MS: 887.5 $[\text{M}+\text{Rb}]^+$; 825.7 $[\text{M}+\text{Na}]^+$; 803.7 $[\text{M}+\text{H}]^+$; 785.7 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$; 767.7 $[\text{M}+\text{H}-2\text{H}_2\text{O}]^+$

^{13}C -NMR (CDCl_3) δ : 214.4 (C16); 175.7 (C2); 169.9 (C10); 168 (C3); 139.1 (C19); 134.7 (C41); 131.3 (C29); 128.2 (C31); 123.4 (C18); 116.7 (C42); 95.5 (C1); 84.2 (C34); 75.2 (C23); 73.4 (C25); 71.5 (C24); 69.5 (C14); 52.9

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(C17); 49.8 (C20); 44.9 (C15); 39.6 (C13); 39.3 (C5); 31.2
(C36); 30.8 (C37); 27.7 (C8); 26.2 (C21); 24.3 (C6); 21.0
(C44); 20.0 (C7); 14.5 (C30); 10.2 (C39)

Example 3

5 17-Allyl-1-(1-thiopropyl)-14-hydroxy-12-[2-(4-hydroxy-3-
methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the compound of Example 2(a) (100mg) and
10 propanethiol (0.1ml) in THF (2ml) and saturated aqueous
sodium hydrogen carbonate solution (2ml) was stirred
vigorously for 24 hours at room temperature. Water (10ml)
was then added and the reaction mixture was extracted with
diethyl ether (20ml). The organic extract was then washed
15 with brine before being dried (MgSO₄), filtered and
evaporated to an oil in vacuo. Chromatography on silica
eluting with hexane/acetone [3:1] then gave the title
compound (42mg) as a foam.

MS: 946 [M+Rb]⁺; 885 [M+Na]⁺; 863 [M+H]⁺;

20 787 [M+H-CH₃(CH₂)₂SH]⁺;

769 [M+H-CH₃(CH₂)₂SH-H₂O]⁺

¹³C NMR (CDCl₃) δ: 212.8 (C16); 191 (C2); 169.3 (C10);
166.7 (C3); 140.8 (C19); 135.2 (C41); 131.3 (C29); 128.7
(C31); 122.3 (C18); 116.8 (C42); 89.6 (C1); 84.1 (C34);
25 73.9 (C25); 73.5 (C35); 70.2 (C14); 56.1 (C9); 51.6 (C17);
48.9 (C20); 44.9 (C15); 39.4 (C13); 38.9 (C5); 36.4 (C40);
33.3 (C26); 31.1 (C36); 30.7 (C37); 29.3 (C8); 28.1 (C21);
27.4 (SCH₂); 24.4 (C6); 21.8 (SCH₂CH₂); 21.0 (C44);

14.3 (C30); 13.6 (S(CH₂)₂CH₃); 10.2 (C39)

Example 4

17-Allyl-1-(N-acetyl)amino-14-hydroxy-12-[2-(4-hydroxy-3-
methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-

5 13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A sample of the first title compound of Example 2 (crude, 100mg) was taken up in methanol (10ml) and acetic anhydride (0.6ml) was added. After being stored at 4°C for 3 days
10 further acetic anhydride (0.3ml) was added and the reaction mixture was stored at this temperature for a further 2 days. The reaction mixture was then poured into saturated aqueous sodium hydrogen carbonate solution (100ml) and this was then extracted with diethyl ether (100ml). The
15 separated organic extract after washing with brine was dried (MgSO₄), filtered and concentrated in vacuo to a foam. Chromatography on silica eluting with dichloromethane/acetone in an increasing acetone gradient then gave material which was further purified by
20 chromatography on silica eluting with ethyl acetate to give the title compound (37mg) as a foam.

MS: 929.1 [M+Rb]⁺; 867.9 [M+Na]⁺; 846 [M+H]⁺; 769.1 [M+H-H₂O-CH₃CONH₂]⁺

¹³C NMR (CDCl₃) δ: 212 (C16); 190.2 (C2); 169.8 (C10);
25 169.4 (CH₃CONH); 163.1 (C3); 140.2 (C19); 135.6 (C41);
132.2 (C29); 129.4 (C31); 122.2 (C18); 116.4 (C42); 87.8 (C1); 84.2 (C34); 76.8 (C12); 76.3 (C23); 74.9 (C24); 70.4 (C14); 52.7 (C17); 51.2 (C9); 47.8 (C20); 45.1 (C15); 44.1

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(C5); 41.6 (C13); 31.3 (C36); 30.6 (C37); 27.3 (C8); 26.0 (C21); 24.3 (C6); 22.9 (CH_3CONH); 21.4 (C7); 18.3 (C44); 16.9 (C47); 15.5 (C43); 14.8 (C30); 9.5 (C39)

5 Further elution then gave the C1 isomeric compound (46mg).

MS: 929.1 $[\text{M}+\text{Rb}]^+$; 867.5 $[\text{M}+\text{Na}]^+$; 845.6 $[\text{M}+\text{H}]^+$; 827.6 $[\text{M}+\text{H}-\text{OH}]^+$; 768.6 $[\text{M}+\text{H}-\text{H}_2\text{O}-\text{CH}_3\text{CONH}_2]^+$

^{13}C NMR (CDCl_3) δ : 210.4 (C16); 194.3 (C2); 169.4 (C10); 169.0 (CH_3CONH); 166.1 (C3); 137.8 (C19); 135.7 (C41); 131.7 (C29); 129.5 (C31); 123.7 (C18); 116.5 (C42); 89.7 (C1); 84.2 (C34); 77.9 (C12); 76.0 (C24); 74.5 (C23); 69.8 (C14); 39.5 (C13); 28.2 (C21); 27.3 (C8); 25.2 (C6); 23.1 (CH_3CONH); 21.5 (C7); 16.9 (C47); 13.2 (C30); 9.9 (C39)

15 Example 5

17-Allyl-1-(N-formylamino-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

20 a) 17-Allyl-14-^tbutyldimethylsilyloxy-12-[2-(4-^tbutyldimethylsilyloxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-1-hydroxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

25 To a solution of FR-900506 (500mg, 0.622mmole) in dry dichloromethane (20ml) at room temperature under nitrogen was added 2,6-dimethylpyridine (0.4ml) and ^tbutyl-dimethylsilyl triflate (362mg, 1.32mmole). After 30

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minutes at room temperature further ^tbutyldimethylsilyl triflate (362mg, 1.32mmole) was added and the reaction mixture was stirred for a further 30 minutes at room temperature. Dichloromethane (30ml) was then added and the
5 reaction mixture was extracted with dilute aqueous hydrochloric acid (25ml) and brine (25ml). The organic extract was dried (MgSO₄), filtered and evaporated to an oil in vacuo. Purification by column chromatography on silica eluting with hexane/acetone [9:1] gave the title
10 compound (606mg, 94%) as an oil.

MS: 1055 [M+Na]⁺; 1117 [M+Rb]⁺

b) 17-Allyl-1-chloro-12-[2-(4-^tbutyldimethylsilyloxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethyl
silyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa
15 -4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

A sample of the compound of step (a) (1g) in dry dichloromethane (10ml) was added dropwise over 5 minutes to a stirred solution of thionyl chloride (0.35ml) and
20 pyridine (0.94ml) in dry dichloromethane (10ml). After stirring for a further 5 minutes at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution (50ml) and this was extracted with diethyl ether (100ml). The separated organic extract
25 after washing with dilute aqueous hydrochloric acid (1M, 50ml), water and brine was then dried (MgSO₄), filtered and concentrated in vacuo to give the subtitle compound as a foam (1g).

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c) 17-Allyl-1-amino-12-[2-(4-^tbutyldimethylsilyloxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

5 A sample of the crude subtitle compound from step (b) (744mg) was dissolved in THF (10ml) and this was then added dropwise to concentrated aqueous ammonia solution (5ml). The reaction mixture after being stirred vigorously for 15 minutes was diluted with water (25ml) and diethyl ether (50ml). The diethyl ether extract was then separated and was washed with brine before being dried (MgSO₄), filtered and concentrated in vacuo to a foam. Chromatography on silica eluting with hexane/ethyl acetate [5:1] then gave the subtitle compound as a foam (250mg).

15 d) 17-Allyl-1-(N-formyl)amino-12-[2-(4-^tbutyldimethylsilyloxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

20 To a crude sample of the subtitle compound from step (c) (120mg) in formic acid (4ml) at room temperature was added acetic anhydride (0.2ml). After stirring for 4 hours at room temperature the reaction was stored at 4°C for 16 hours before being poured into saturated aqueous sodium hydrogen carbonate solution (100ml). After stirring this mixture for 20 minutes at room temperature it was extracted with diethyl ether (50ml) and this extract was then washed

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with brine before being dried (MgSO_4), filtered and concentrated in vacuo to give the subtitle compound as an oil.

e) 17-Allyl-1-(N-formyl)amino-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A crude sample of the subtitle compound from step (d) (120mg) was taken up in methanol (3ml) and aqueous hydrofluoric acid was added (0.2ml). After 2 hours at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution (20ml) and this was then extracted with diethyl ether (40ml). The separated organic extract was then washed with brine before being dried (MgSO_4), filtered and concentrated in vacuo to a foam. Chromatography on silica eluting with hexane/acetone [2:1] then gave the title compound (30mg) as a foam.

MS: 915.2 $[\text{M}+\text{Rb}]^+$; 831.6 $[\text{M}+\text{H}]^+$; 813.6 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$; 768.6 $[\text{M}+\text{H}-\text{H}_2\text{O}-\text{H}_2\text{NCHO}]^+$
¹³C NMR (CDCl_3) δ : 214.6 (C16); 193.6 (C2); 169.2 (C10); 166.1 (C3); 159.9 (NHCOH); 137.2 (C19); 135.3 (C41); 122.6 (C18); 116.5 (C42); 88.7 (C1); 84 (C34); 77.9 (C12); 69.2 (C14); 56.2 (C9); 48.7 (C20); 43.6 (C15); 39.9 (C13); 24.2 (C6); 20.8 (C44); 16.7 (C47); 14.7 (C43); 14.0 (C30); 11.1 (C39)

Example 6

17-Allyl-1-fluoro-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclo

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hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

To a cold (0°C) solution of the subtitle compound of
5 Example 5(a) (250mg) in dry dichloromethane (10ml) under
nitrogen was added diethylaminosulphur trifluoride
(100mg). After stirring for 2 hours at 0°C the reaction
mixture was poured into saturated aqueous sodium hydrogen
carbonate solution (30ml) and this was then extracted with
10 diethyl ether (100ml). The separated organic extract after
washing with brine was dried (MgSO₄), filtered and
concentrated in vacuo to a foam (248mg). This was then
dissolved in acetonitrile (10ml) and 40% aqueous
hydrofluoric acid (0.2ml) was added. After being stirred
15 for two hours at room temperature the reaction mixture was
poured into saturated aqueous sodium hydrogen carbonate
solution (50ml) and this was then extracted with diethyl
ether (100ml). The separated organic extract was then
washed with brine and was dried (MgSO₄), filtered and
20 concentrated in vacuo to an oil. Chromatography on silica
eluting with dichloromethane/acetonitrile [2:1] then gave
the title compound (28mg) as a foam.

MS: 890.5 [M+Rb]⁺; 828.9 [M+Na]⁺; 787 [M+H-HF]⁺; 769
[M+H-HF-H₂O]⁺

25 ¹⁹F NMR δ: -139.55 (d, J=28.15Hz); -141.55 (d, J=28.15Hz)
(two rotamers)

¹³C NMR (CDCl₃) δ: 211.9 (C16); 192.3 (C2); 169 (C10);
164.2 (C3); 140 (C19); 135.6 (C41); 132 (C29); 129.5 (C31);

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122.8 (C18); 116.5 (C42); 112.8 (C1); 84.2 (C34); 77.2 (C12); 76.0 (C23); 75.1 (C25); 73.5 (C35); 72.5 (C24); 69.8 (C14); 48.1 (C20); 45 (C5); 43.8 (C15); 40.8 (C13); 32.3 (C26); 31.2 (C36); 30.7 (C37); 26.8 (C8); 25.9 (C21); 25.0 (C6); 21.7 (C7); 19.4 (C44); 15.8 (C47); 15.1 (C43); 14.5 (C30); 9.7 (C39)

Example 7

The first title compound of Example 2 was tested in Test D, and found to inhibit IL-2 secretion by 50% (IC₅₀) at a concentration of 2×10^{-10} M.

Example 8

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

(a) 17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product from Example 5(a) (1.28g) in methanol (100ml) containing pyridinium p-toluene sulphonate was stirred for 18 hours at room temperature. Volatiles were then removed in vacuo and the residue was dissolved in diethyl ether. The ethereal solution after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate solution and brine was dried (MgSO₄), filtered and evaporated in vacuo to give the

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subtitle compound as a pale yellow foam (0.97g).

(b) 17-Allyl-1-hydroxy-12-[2-(4-trifluoromethylsulphonyloxy
-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethyl
silyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa
5 -4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

To a cold (-10°C) stirred solution of the product of step
(a) (0.97g) in dry dichloromethane (25ml) under nitrogen
was added trifluoromethanesulphonic anhydride (0.1ml).
10 After stirring for 15 minutes at -10°C, saturated aqueous
sodium hydrogen carbonate solution was added and the
reaction mixture was extracted with diethyl ether. The
ether extracts were then washed with saturated aqueous
sodium hydrogen carbonate solution, dilute aqueous
15 hydrochloric acid (1N), saturated aqueous sodium hydrogen
carbonate solution and brine before being dried (MgSO₄),
filtered and concentrated in vacuo to give the title
compound as an oil (0.95g).

(c) 17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)
20 -1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Silica (55g, Merck Kieselgel 60) was added to a solution of
the product from step (b) (0.9g) in dichloromethane
25 (250ml). Volatiles were then removed in vacuo at room
temperature and the resulting freely flowing powder was
stored at 8°C for 16 hours. The support was then washed
with ethyl acetate and 10% acetone in ethyl acetate

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containing 2,6-dimethylpyridine. The combined organic extracts after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate solution and brine were dried (MgSO_4), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane in an acetone gradient then gave the title compound (0.126g) as a foam.

(d) 17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the compound of step (c) (25mg) in acetonitrile (5ml) was added 40% aqueous hydrofluoric acid (1ml). After stirring for 1 hour at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with diethyl ether. The organic extract was then dried, (MgSO_4), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane [1:2] then gave the title compound (18mg) as a foam.

Example 9

1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 8 (15mg) in methanol (4ml) was added Pd-on-C (4mg, 10%) and the

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resulting suspension was then stirred in an atmosphere of hydrogen for 1 hour at 0°C. The reaction mixture was then filtered and volatiles were removed in vacuo. Chromatography on silica then gave the title compound as a
5 foam (13mg).

Example 10

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
10 2,3,10,16-tetraone

(a) 17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

15 To a solution of the product of Example 8(c) (170mg) in dry THF (15ml) at -70°C was added a solution of L-selectride in THF (1M) slowly under nitrogen until no starting material remained (0.4ml). Saturated aqueous ammonium chloride solution (0.5ml) was then added at -70°C followed by
20 ~~aqueous hydrogen peroxide solution (30% by weight, 1ml)~~ and ethanolamine (0.1ml). After warming to 0°C the reaction mixture was extracted with diethyl ether and this was washed with water (x2), dilute aqueous hydrochloric acid (1N) and saturated aqueous sodium hydrogen carbonate
25 solution, before being dried (MgSO₄), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane [2:7] then gave the title compound (151mg) as a foam.

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MS (FAB): 911 [M+Na]⁺; 972 [M+Rb]⁺.

(b) 17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of step (a) (150mg) in acetonitrile (20ml) was added 40% aqueous hydrofluoric acid (3ml). After stirring for 1 hour at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with diethyl ether. The organic extracts were then dried, (MgSO₄), filtered and evaporated to an oil in vacuo.

Chromatography on silica eluting with acetone/hexane [1:3] then gave the title compound (130mg) as a foam.

MS (plasma spray): 738.54 [M+H-2H₂O]⁺; 756.58 [M+H-H₂O]⁺; 774.6 [M+H]⁺; 791.57 [M+NH₄]⁺
¹³C NMR (CDCl₃) δ: (Major rotamer) 212.5 (C16); 196.2 (C2); 169 (C10); 164.7 (C3); 138.8 (C19); 135.5 (C40); 131.4 (C31); 131 (C29); 122.4 (C18); 116.5 (C41); 97 (C1); 77.7 (C12); 75 (C23); 69.9 (C14); 67 (C37); 56.5 (C9); 48.5 (C20); 43.6 (C15); 27.6 (C8); 26 (C21); 24.4 (C6); 20.9 (C7); 20.3 (C43); 13.9 (C30); 9.5 (C38)

Example 11

1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the title compound of Example 10 (22mg) in

methanol (10ml) was added 10% Pd-on-C (5mg) and the resulting suspension was then stirred in an atmosphere of hydrogen for 2 hours at 0°C. The reaction mixture was then filtered and volatiles were removed in vacuo. Chromatography on silica then gave the title compound as a foam (18mg).

MS (plasma spray): 794 $[M+NH_4]^+$

Example 12

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-carboxylic acid)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 8(c) (393mg) in ^tbutanol (30ml) containing 1-methylcyclohex-1-ene (4ml) was added dropwise a solution of sodium chlorite (0.75g) and sodium phosphate (0.75g) in distilled water (10ml). After stirring for 10 minutes at room temperature the reaction mixture was partitioned between ethyl acetate and water and the organic extract was separated. This was then washed with aqueous sodium phosphate solution, an aqueous sodium thiosulphate/sodium phosphate mixture and aqueous sodium phosphate solution before being dried (MgSO₄), filtered and evaporated in vacuo to give the title compound (350mg) as a foam.

Example 13

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-

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18-ene-2,3,10,16-tetraone

To a solution of the product of Example 12 (350mg) in acetonitrile (30ml) was added 40% aqueous hydrofluoric acid (3ml). After stirring for 1.5 hours at room temperature the reaction mixture was poured into ethyl acetate and the organic extract was washed with water and saturated aqueous sodium phosphate solution (x4) before being dried (MgSO₄), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane/acetic acid [40:10:1] then gave the title compound (32mg) as a foam.

MS (FAB): 771.02 [M-OH+H]⁺; 811 [M+Na]⁺; 872.72 [M+Rb]⁺

¹³C NMR δ: (Major rotamer) 212.6 (C16); 196.1 (C2); 181.6 (C37); 169.1 (C10); 164.7 (C3); 138.9 (C19); 135.6 (C40); 132.7 (C29); 130.3 (C31); 122.6 (C18); 116.7 (C41); 98.6 (C1); 77.8 (C12); 75.3 (C23); 73.6 (C25); 72.6 (C24); 70.0 (C14); 56.7 (C9); 52.9 (C17); 48.7 (C20); 26.3 (C21); 24.6 (C6); 21.1 (C7); 20.4 (C43); 14.1 (C30); 9.7 (C38).

20 Example 14

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid methyl ester)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

25 To a solution of the product of Example 13 (25mg) in diethyl ether (5ml) at 0°C was added diazomethane. Volatiles were then removed in vacuo to give the title compound as a foam (25mg).

Example 15

1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid methyl ester)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
5 18-ene-2,3,10,16-tetraone

To a solution of the product of Example 14 (20mg) in methanol (10ml) was added 10% Pd-on-C (4mg) and the resulting suspension was then stirred in an atmosphere of hydrogen for 2 hours at 0°C. The reaction mixture was then
10 filtered and volatiles were removed in vacuo. Chromatography on silica then gave the title compound as a foam (17mg).

Example 16

1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid)
15 -1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

To a solution of the product of Example 15 (18mg) in methanol (10ml) was added 10% Pd-on-C (4mg) and the
20 ~~resulting suspension was then stirred in an atmosphere of~~
~~hydrogen for 2 hours at 0°C. The reaction mixture was then~~
~~filtered and volatiles were removed in vacuo.~~
Chromatography on silica then gave the title compound as a foam (17mg).

25 MS (FAB): 874 [M+Rb]⁺

Example 17

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-methyl
propenoate)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-

23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of Example 8(d) (140mg) and (carbomethoxymethylene)triphenylphosphorane (140mg) in dry distilled toluene (10ml) was stirred and heated at 70°C for one hour. After stirring at room temperature overnight the reaction mixture was diluted with diethyl ether and this was then washed with saturated aqueous sodium hydrogen carbonate solution and brine. The organic extract was then dried (MgSO₄), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing diethyl ether gradient then gave the title compound (70mg) as a foam.

Example 18

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-methyl propenoate)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 17 (70mg) in acetonitrile (10ml) was added 40% aqueous hydrofluoric acid (1ml). After stirring for 1 hour at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with diethyl ether. The combined ether extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, were dried (MgSO₄), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the

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title compound (55mg) as a foam.

MS (plasma spray): 792.78 $[M+H-2H_2O]^+$; 810.80

$[M+H-H_2O]^+$; 828.86 $[M+H]^+$; 845.84 $[M+NH_4]^+$

MS (negative plasma spray): 826.09 $[M-H]^+$

5 1H NMR ($CDCl_3$) δ : 6.93 (1H, dd, $J=8.1$ and 16.6 Hz);
5.78 (1H, d, $J=5.78$ Hz), 3.71 (3H, s, CO_2Me)

^{13}C NMR δ : (Major rotamer) 212.4 (C16); 196.1 (C2);
153.3 (C38); 138.8 (C19); 135.4 (C43); 122.6 (C18); 119
(C37); 116.6 (C44); 97.1 (C1); 56.6 (C9); 51.3 (C40); 9.7

10 (C41).

Example 19

1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methyl
vinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-

15 2,3,10,16-tetraone

(a) 1-Hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methyl
vinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-17-ethyl-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

20 ~~The subtitle compound was prepared from FR-900520 in a~~
~~manner analogous to the compound of Example 8(c).~~

(b) 1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-
methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-

25 18-ene-2,3,10,16-tetraone

The product of step (a) was deprotected following the
method of Example 8(d) to give the title compound.

Example 20

1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-
-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-
dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

5 The product of Example 19 was reduced by the method of Example 10(a) to give the title compound.

MS (plasma spray): 779 [M+NH₄]⁺

Example 21

1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid)
-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

Oxidation of the product of Example 19(a) following the method of Example 12 and then deprotection following the
 15 method of Example 13 gave the title compound.

MS (FAB): 709 [M+Na]⁺

Example 22

1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid methyl
ester)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
20 tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

Esterification of the product of Example 21 following the
 method of Example 14 yielded the title compound.

Example 23

25 1,14-dihydroxy-12-[2-(cyclopentyl-3-methyl propenoate)-1-
methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetra
methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

Wittig reaction on the product of Example 19(a) following the method of Example 17 and then deprotection following the method of Example 18 gave the title compound.

MS (plasma spray): 834 $[M+NH_4]^+$

5 Example 24

1-Hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

- 10 a) 1-Hydroxy-12-[2-(4-trifluoromethylsulphonyloxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a cold (-10°C), stirred solution of 1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (Example 12, WO 89/05304) (0.3g) in dry dichloromethane (12ml) under nitrogen was added trifluoromethanesulphonic anhydride (0.1ml) until no starting material remained. Saturated aqueous sodium hydrogen carbonate solution was then added and the reaction mixture was extracted with diethyl ether. The ether extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), and saturated aqueous sodium hydrogen carbonate solution, were dried (MgSO₄), filtered and concentrated in vacuo to give the title compound as an oil (300mg).

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b) 1-Hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

5 Silica (18g, Merck Kieselgel 60) was added to a solution of the product of step (a) (300mg) in dichloromethane (100ml). Volatiles were then removed in vacuo at room temperature and the resulting freely flowing powder was stored at 8°C for 16 hours. The support was then washed with acetone
10 containing triethylamine and the solvent was evaporated in vacuo to an oil. Chromatography on silica eluting with hexane in an acetone gradient then gave the title compound as a foam (51mg).

Example 25

15 1-Hydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Reduction of the product of Example 24 following the method
20 of Example 10(a) yielded the title compound.

Example 26

1-Hydroxy-12-[2-(cyclopentyl-3-carboxylic acid)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone
25

Oxidation of the product of Example 24 using the method of Example 12 gave the title compound.

Example 27

1-Hydroxy-12-[2-(cyclopentyl-3-carboxylic acid methylester)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

- 5 Esterification of the product of Example 18 using diazomethane following the method of Example 14 gave the title compound.

Example 28

1-Hydroxy-12-[2-(cyclopentyl-3-methyl propenoate)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Wittig reaction with the product of Example 24 following the method of Example 17 yielded the title compound.

15 Example 29

1-Hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

20 (a) 1-Hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-14,18-ene-2,3,10,16-tetraone

1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (FR-900520) (100mg) and p-toluenesulphonic acid (2mg) were dissolved in dry toluene (20ml) and were heated

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for 2 hours at 100°C under an atmosphere of nitrogen. Removal of solvent in vacuo and chromatography on silica eluting with hexane/acetone [2:1] gave the sub-title compound as a foam (80mg).

5 MS (FAB): 774.8 [M+H]⁺; 796.85 [M+Na]⁺; 858.71 [M+Rb]⁺.

¹³C NMR δ: (major rotamer) 201.15 (C16); 196.0 (C2); 169.2 (C10); 165.1 (C3); 147.8 (C15); 138.0 (C19); 123.82 (C18); 97.88 (C1); 84.05 (C34).

10 (b) 1-Hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A sample of the product from step (a) was dissolved in 15 methanol (20ml) and 10% Pd-on-carbon (10mg) was added. The mixture was stirred in an atmosphere of hydrogen for 1.5 hours at room temperature and pressure, and was then filtered through celite and evaporated to an oil in vacuo. Column chromatography on silica eluting with hexane/acetone 20 [2:1] gave the subtitle compound as a foam (50mg).

MS (FAB): 776 [M+H]⁺; 798 [M+Na]⁺; 860 [M+Rb]⁺.

¹³C NMR δ: (major rotamer) 212.34 (C16); 196.42 (C2); 169.38 (C10); 165.16 (C3); 138.9 (C19); 124.16 (C18); 97.41 (C1); 84.19 (C34).

25 c) 1-Hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The title compound was prepared from the product of step (b) using the method of Example 1.

Example 30

1-Hydroxy-12-[2-(cyclopentyl-3-methanol)-1-methylvinyl]-
5 23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-
dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

Reduction of the product of Example 29 using the method of Example 10(a) yielded the title compound.

10 Example 31

1-Hydroxy-12-[2-(cyclopentyl-3-carboxylic acid)-1-methyl
vinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-
11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

15 Oxidation of the product from Example 29 following the method of Example 12 gave the title compound.

Example 32

1-Hydroxy-12-[2-(cyclopentyl-3-carboxylic acid methyl
ester)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
20 tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

Esterification of the product of Example 31 using the method of Example 14 yielded the title compound.

Example 33

25 1-Hydroxy-12-[2-(cyclopentyl-3-methyl propenoate)-1-
methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

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Wittig reaction of the product of Example 29 following the method of Example 17 gave the title compound.

Example 34

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-carboxaldehyde)
5 -1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

The title compound was prepared from 17-allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-
10 dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (Example
17, WO 89/05304) using the method of Example 8(c).

Example 35

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-methanol)-1-
15 methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-
dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

Reduction of the product from Example 34 following the method of Example 10(a) gave the title compound.

20 Example 36

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-carboxylic acid)
-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

25 Oxidation of the product of Example 34 following the method of Example 12 yielded the title compound.

Example 37

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-carboxylic acid

methyl ester)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Esterification of the product of Example 36 using the method of Example 14 gave the title compound.

Example 38

17-Allyl-1-hydroxy-12-[2-(cyclopentyl-3-methyl propenoate)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Wittig reaction of the product of Example 34 following the method of Example 17 yielded the title compound.

Example 39

17-Allyl-1,14-dihydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

(a) 17-Allyl-1-hydroxy-12-[2-(4-^tbutyldimethylsilyloxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The subtitle compound was prepared as in Example 5(a) (1.28g).

(b) 17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

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A solution of the product from step (a) in methanol (100ml) containing pyridinium p-toluene sulphonate was stirred for 18 hours at room temperature. Volatiles were then removed in vacuo and the residue was dissolved in diethyl ether.

5 The ethereal solution after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate solution and brine was dried (MgSO₄), filtered and evaporated in vacuo to give the subtitle compound as a
10 pale yellow foam (0.97g).

(c) 17-Allyl-1-hydroxy-12-[2-(4-trifluoromethylsulphonyloxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-

15 tetraone

To a cold (-10°C) stirred solution of the product of step (b) (0.97g) in dry dichloromethane (25ml) under nitrogen was added trifluoromethanesulphonic anhydride (0.1ml). After stirring for 15 minutes at -10°C saturated aqueous
20 sodium hydrogen carbonate solution was added and the reaction mixture was extracted with diethyl ether. The ether extracts were then washed with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen
25 carbonate solution and brine before being dried (MgSO₄), filtered and concentrated in vacuo to give the title compound as an oil (0.95g).

(d) 17-Allyl-1-hydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclo

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hexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-
dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Silica (55g, Merck Kieselgel 60) was added to a solution of
5 the product of step (a) (0.9g) in dichloromethane (250 ml).
Volatiles were then removed in vacuo at room temperature
and the resulting freely flowing powder was stored at 8°C
for 16 hours. The support was then washed with ethyl
acetate and 10% acetone in ethyl acetate containing
10 2,6-dimethyl pyridine. The combined organic extracts after
washing with saturated aqueous sodium hydrogen carbonate
solution, dilute aqueous hydrochloric acid (1N), saturated
aqueous sodium hydrogen carbonate solution and brine were
dried (MgSO₄), filtered and concentrated to an oil in
15 vacuo. Chromatography on silica eluting with hexane in an
acetone gradient then gave the title compound (0.28g) as a
foam.

(e) 17-Allyl-1,14-dihydroxy-12-(2-(4(S)-hydroxy-3-methoxy
cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
20 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
ene-2,3,10,16-tetraone

To a solution of the product of step (d) (0.28g) in
acetonitrile (10 ml) was added 40% aqueous hydrofluoric
acid (2ml). After stirring for 1 hour at room temperature
25 the reaction mixture was poured into saturated aqueous
sodium hydrogen carbonate solution and the mixture was
extracted with diethyl ether. The organic extract was then
dried (MgSO₄), filter d and evaporated to an oil in

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vacu. Chromatography on silica eluting with acetone/hexane [1:2] then gave the title compound (0.22g) as a foam.

MS (FAB): 888.43 [M+Rb]⁺

5 ¹³C NMR (CDCl₃) δ: (Major rotamer) 212.4 (C16); 196.1 (C2); 168.9 (C10); 164.6 (C3); 138.8 (C19); 135.4 (C41); 132.3 (C29); 128.9 (C31); 122.3 (C18); 116.4 (C42); 96.8 (C1); 81.9 (C34); 77.4 (C12); 75 (C23); 73.5 (C25); 72.7 (C24); 56.8 (C9); 52.7 (C17); 48.4 (C 20); 43.3 (C15); 10 39.6 (C13); 39.1 (C5); 35.6 (C21); 34.6 (C27); 30.4 (C32); 20.9 (C7); 20.2 (C44); 13.7 (C30); 9.4 (C39).

Example 40

1,14-Dihydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetra
15 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
ene-2,3,10,16-tetraone

a) 1-Hydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-1-
methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-17-
ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
20 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Using the method of Example 39(a)-(d) the subtitle compound was prepared from 1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxy cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
25 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (FR-900520).

b) 1,14-Dihydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)
-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-

tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

Using the method of Example 39(e) the title compound was prepared from the product of step (a).

5 MS (FAB): 876 [M+Rb]⁺

Example 41

1,14-dihydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-1-
methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetra
methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
10 ene-2,3,10,16-tetraone

To a solution of the product of Example 39 (20mg) in methanol (10ml) was added 10% Pd-on-C (5mg) and the resulting suspension was then stirred in an atmosphere of hydrogen for 2 hours at 0°C. The reaction mixture was then
15 filtered and volatiles were removed in vacuo. Chromatography on silica then gave the title compound as a foam (16mg).

MS (FAB): 890 [M+Rb]⁺

Example 42

20 17-Allyl-1,14-dihydroxy-12-[2-(4-iodo-3-methoxycyclohexyl)
-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

a) 17-Allyl-1-hydroxy-12-[2-(4-iodo-3-methoxycyclohexyl)
25 -1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a stirred, cold (-20°C) solution of the product of

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Example 39(d), (0.1g) in dry distilled dichloromethane (5ml) containing dry pyridine (0.4ml) under nitrogen was added trifluoromethanesulphonic anhydride (0.3ml). After 20 minutes at -20°C 2ml of saturated aqueous sodium hydrogen carbonate solution was added and the reaction mixture was extracted with diethyl ether. The organic extracts were then washed with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N) and saturated aqueous sodium hydrogen carbonate solution before being dried (MgSO₄), filtered and concentrated to an oil in vacuo. This was taken up in dry benzene (10ml) containing triethylamine (0.1ml) and was heated under reflux for one hour. Tetra-ⁿbutylammonium iodide (200mg) was then added and heating was continued for a further 30 minutes. The reaction mixture was then cooled and poured into ether. The separated ether layer was washed with dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate, sodium thiosulphate solution and brine, before being dried (MgSO₄), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing acetone gradient gave the subtitle compound (30mg) as a foam.

b) 17-Allyl-1,14-dihydroxy-12-[2-(4-iodo-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of step (a) (30mg) in acetonitrile (7ml) was added 40% aqueous hydrofluoric acid

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(1ml). After stirring for 1 hour at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with diethyl ether. The combined ether extracts were then washed with saturated aqueous sodium hydrogen carbonate solution and brine before being dried (MgSO_4), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane [1:4] then gave the title compound (17mg) as a foam.

10 MS (FAB): 870.74 $[\text{M}-\text{I}+\text{Rb}]^+$; 997.15 $[\text{M}+\text{Rb}]^+$
 ^{13}C NMR (CDCl_3) δ : (Major rotamer) 213 (C16); 196.3 (C2) 169.1 (C10); 164.8 (C3); 139.0 (C19); 135.7 (C41); 132.8 (C29); 129.1 (C31); 122.4 (C18); 116.7 (C18); 97 (C1); 78.9 (C34); 76.6 (C12); 75.2 (C23); 73.8 (C25); 73.0 (C24); 70.2 (C14); 56.7 (C9); 52.8 (C17); 26.3 (C21); 9.4 (C39).

Example 43

17-Allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

a) 17-Allyl-1-hydroxy-12-[2-(4-(imidazol-1-yl)(thiocarbonyl)oxy)-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of Example 39(b) (280mg) in dry distilled dichloroethane (40ml) containing

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1,1'-thiocarbonyldiimidazole (2g) was heated under reflux for 36 hours under an atmosphere of nitrogen. Volatiles were then removed in vacuo and the residue was chromatographed on silica eluting with
5 dichloromethane/acetone [9:1] to give the subtitle compound (105mg) as a foam.

b) 17-Allyl-1,2-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
10 [22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

A solution of the product of step (a) (105mg) in dry
~~benzene~~ (25ml) containing AIBN (2,2'-bisisobutyronitrile) (3mg) was heated to 40°C under nitrogen. Tributyltin
hydride (0.1ml) was then added dropwise by syringe. The
15 temperature was then raised to 60°C over 5 minutes and a further 0.1 ml of tributyltin hydride was added. The temperature was then further raised to 90°C over 10 minutes and an additional 0.1ml of tributyltin hydride was added. After a further 10 minutes no starting material remained
20 and volatiles were removed in vacuo after cooling to room temperature. ~~Chromatography on silica then gave the subtitle compound as an oil~~ (85mg).

c) 17-Allyl-1-hydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-
25 13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of step (b) (85mg) in glacial acetic acid (10ml) containing copper (II) acetate (1g) was

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heated at 80°C for 5 minutes. The cooled reaction mixture was then poured into saturated aqueous sodium hydrogen carbonate solution and this was extracted with diethyl ether. The ether extracts were then dried (MgSO₄),
 5 filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane [2:5] then gave the subtitle compound as a foam (40mg).

d) 17-Allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of step (c) (40mg) in acetonitrile (8ml) was added 40% aqueous hydrofluoric acid (1ml). After stirring for 1 hour at room temperature the
 15 reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with diethyl ether. The ether extracts were then dried (MgSO₄), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane in an
 20 ~~increasing acetone gradient then gave the title~~ compound as a foam (20mg).

MS (plasma spray): 752.73 [M+H-2H₂O]⁺; 770.76 [M+H-H₂O]⁺; 788.77 [M+H]⁺; 805.79 [M+NH₄]⁺
¹³C NMR (CDCl₃) δ: (Major rotamer) 212.9 (C16);
 25 196.2 (C2); 169 (C10); 164.7 (C3); 139.0 (C19); 135.6 (C41); 131.6 (C29); 130.5 (C31); 122.4 (C18); 116.7 (C42);
 97 (C1); 78.9 (C34); 77 (C12); 75.2 (C23); 73.7 (C25); 72.8 (C24); 70.1 (C14); 56.4 (C9); 52.7 (C17); 48.5 (C20); 43.1

(C15); 39.7 (C13); 39.2 (C5); 26.3 (C21); 21.2 (C7); 20.5 (C44); 14.1 (C30); 9.4 (C39).

Example 44

1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-
5 23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-
dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

a) 1-Hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-
methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-
10 17-ethyl-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The subtitle compound was prepared from 1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxo-4-
15 azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (FR-900520) following the method of Example 5(a) and 39(b).

b) 1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-
methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-
20 18-ene-2,3,10,16-tetraone

The title compound was prepared from the product of step (a) following the method of Example 43.

MS (plasma spray): 794 [M+NH₄]⁺

Example 45

25 1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-
23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-
dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

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To a solution of the product of Example 43 (28mg) in methanol (10ml) was added 10% Pd-on-C (5mg) and the resulting suspension was then stirred in an atmosphere of hydrogen for 2 hours at 0°C. The reaction mixture was then
5 filtered and volatiles were removed in vacuo. Chromatography on silica then gave the title compound as a foam (25mg).

MS (plasma spray): 808 $[M+NH_4]^+$

Example 46

10 17-Allyl-1-hydroxy-12-[2-(3-methoxycyclohexyl)-1-methyl
vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4
-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The title compound was prepared from 17-allyl-1-hydroxy-12-
[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-
15 dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (Example
17, WO 89/05304) following the method of Example 43.

Example 47

1-Hydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-
-20-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

---The title compound was prepared from 1-hydroxy-12-[2-
(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-
dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
25 azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone
(Example 12, WO 89/05304) following the method of Example
43.

Example 48

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1-Hydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-
dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

a) 17-Ethyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclo
 5 hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
14,18-diene-2,3,10,16-tetraone
 17-Ethyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclo
 hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
 10 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
 ene-2,3,10,16-tetraone (FR-900520) (100mg) and
 p-toluenesulphonic acid (2mg) were dissolved in dry toluene
 (20ml) and were heated for 2 hours at 100°C under an
 atmosphere of nitrogen. Removal of solvent in vacuo and
 15 chromatography on silica eluting with hexane/acetone [2:1]
 gave the sub-title compound as a foam (80mg).

MS (FAB): 774.8 [M+H]⁺; 796.85 [M+Na]⁺; 858.71
 [M+Rb]⁺.

¹³C NMR δ: (major rotamer) 201.15 (C16); 196.0 (C2);
 20 169.2 (C10); 165.1 (C3); 147.8 (C15); 138.0 (C19); 123.82
 (C18); 97.88 (C1); 84.05 (C34).

b) 17-Ethyl-1-hydroxy-12-[2-(4-hydroxy-3-
methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
 25 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A sample of the product from step (a) was dissolved in
 methanol (20ml) and 10% Pd-on-carbon (10mg) was added. The
 mixtur was stirred in an atmosphere of hydrogen for 1.5

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hours at room temperature and pressure, and was then filtered through celite and evaporated to an oil in vacuo. Column chromatography on silica eluting with hexane/acetone [2:1] gave the title compound as a foam (50mg).

5 MS (FAB): 776 [M+H]⁺; 798 [M+Na]⁺; 860 [M+Rb]⁺.

¹³C NMR δ: (major rotamer) 212.34 (C16); 196.42 (C2); 169.38 (C10); 165.16 (C3); 138.9 (C19); 124.16 (C18); 97.41 (C1); 84.19 (C34).

c) 1-Hydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-
 10 23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-
dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

The title compound was prepared from the product of step (b) following the method of Example 43.

15 Example 49

17-Allyl-1,14-dihydroxy-12-[2-(cyclohex-3-enyl)-1-
methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

~~20 a) 17-Allyl-1-hydroxy-12-[2-(4-iodo-3-methoxycyclohexyl)~~
~~-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-~~
~~13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo~~
~~[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone~~

To a stirred, cold (-20°C) solution of 17-allyl-1-hydroxy-
 25 12-[2-(4S)-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14-
^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-
 tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-
 18-ene-2,3,10,16-tetraon [the product of Example 39(d)]

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(0.54g) in dry distilled dichloromethane (25ml) containing dry pyridine (2ml) under nitrogen was added trifluoromethanesulphonic anhydride (1.2ml). After 20 minutes at -20°C 10ml of saturated aqueous sodium hydrogen carbonate solution was added and the reaction mixture was extracted with diethyl ether. The organic extracts after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N) and saturated aqueous sodium hydrogen carbonate solution were dried (MgSO₄), filtered and concentrated to an oil in vacuo. This was taken up in dry benzene (30ml) containing dry pyridine (0.3ml) and tetra-ⁿbutylammonium iodide (1.0g) was added. After heating for 30 minutes under reflux the reaction mixture was cooled to room temperature and poured into ether. The separated ether layer was washed with dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate, sodium thiosulphate solution and brine, before being dried (MgSO₄), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with acetone/hexane-[1:4] then gave the title compound (500mg) as a diastereoisomeric mixture of iodides. [A smaller scale synthesis of the subtitle compound was described in Example 42(a)].

b) 17-Allyl-1,2-dihydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

To a solution of the product of step (a) (500mg) in glacial

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acetic acid (8ml) was added zinc dust. After stirring for 10 minutes at room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and this was extracted with diethyl ether. The ether extracts were then washed with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N) and saturated aqueous sodium hydrogen carbonate solution before being dried (MgSO_4), filtered and concentrated in vacuo to give the subtitle compound (320mg) as an oil.

c) 17-Allyl-1-hydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of step (b) (320mg) in glacial acetic acid (8ml) containing copper (II) acetate was heated at 85°C for 5 minutes. After cooling to room temperature the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and this was then extracted with diethyl ether. The organic extract after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N) and saturated aqueous sodium hydrogen carbonate solution was dried (MgSO_4), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the title compound as a foam (280mg).

d) 17-Allyl-1,14-dihydroxy-12-[2-(cyclohex-3-enyl)-1-

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methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

To a solution of the product from step (c) (280mg) in
5 acetonitrile (20ml) was added 40% aqueous hydrofluoric acid
(4ml). After stirring for 30 minutes at room temperature
the reaction mixture was poured into saturated aqueous
sodium hydrogen carbonate solution and the mixture was
extracted with diethyl ether. The combined ether extracts
10 after washing with saturated aqueous sodium hydrogen
carbonate solution were then dried (MgSO₄), filtered and
concentrated to an oil in vacuo. Chromatography on silica
eluting with hexane in an increasing acetone gradient then
gave the title compound as a foam (0.227g).

15 MS (plasma spray): 720.52 [M+H-2H₂O]⁺; 738.50
[M+H-H₂O]⁺; 756.58 [M+H]⁺; 773.53 [M+NH₄]⁺

MS (FAB): 840.81 [M+Rb]⁺

¹³C NMR δ: (Major rotamer) 212.5 (C16); 196.2 (C2);
168.9 (C10); 164.6 (C3); 138.8 (C19); 135.5 (C40); 131.4
20 (C31); 131.2 (C29); 126.9 (C34); 125.9 (C35); 122.4 (C18);
116.5 (C41); 96.9 (C1); 77.4 (C12); 76.5 (C23); 73.5 (C25);
72.7 (C24); 69.9 (C14); 56.5 (C9); 52.7 (C17); 48.5 (C20);
43.4 (C15); 26.1 (C21); 20.3 (C43); 13.8 (C30); 9.4 (C38).

Example 50

25 1,14-Dihydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-23,25-
dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The title compound was prepared from the subtitle compound

of Example 40(a) using the method of Example 49.

MS (FAB): 829 [M+Rb]⁺.

Example 51

1,14-dihydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-23,25-
 5 dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

a) 1-Hydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-
1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-
17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
 10 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The subtitle compound was prepared from 1,14-dihydroxy-
 12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-
 dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
 azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone
 15 (Example 10, WO 89/05304) following the method of Example
 39(a)-(d).

b) 1,14-dihydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-
23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-
dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
 20 tetraone

The title compound was prepared from the product of step
 (a) following the method of Example 49.

MS (FAB): 843 [M+Rb]⁺

Example 52

25 17-Allyl-1-hydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-
23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

(a) 17-Allyl-1-hydroxy-12-[2-(4(S)-hydroxy-3-

methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The subtitle compound was prepared from 17-allyl-1-
 5 hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]
 -23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-aza
 tricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone
 (Example 17, WO 89/05304) following the method of Example
 39(a)-(d).

10 (b) 17-Allyl-1-hydroxy-12-[2-(cyclohex-3-enyl)-1-methyl
vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4
-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The title compound was prepared from the product of step
 (a)-following the method of Example 49(a)-(c).

15 Example 53

1-Hydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-23,25-
dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

a) 1-Hydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-
 20 1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

The subtitle compound was prepared from 1-hydroxy-
 12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-
 25 dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-aza
 tricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone [the
 product of Example 48(b)] following the method of Example
 39(a)-(d).

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MS (FAB): 861 [M+Rb]⁺

b) 1-Hydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

5 The title compound was prepared from the product of step (a) following the method of Example 49(a)-(c).

Example 54

1-Hydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-

10 azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

a) 1-Hydroxy-12-[2-(4-trifluoromethylsulphonyloxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

15 To a cold (-10°C) stirred solution of 1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (Example 12, WO 89/05304) (0.3g)

20 in dry dichloromethane (12ml) under nitrogen was added trifluoromethanesulphonic anhydride (0.1ml) until no starting material remained. Saturated aqueous sodium hydrogen carbonate solution was then added and the reaction mixture was extracted with diethyl ether. The ether
25 extracts, after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), and saturated aqueous sodium hydrogen carbonate solution, were dried (MgSO₄), filtered and concentrated

in vacuo to give the subtitl compound as an oil (300mg).

b) 1-Hydroxy-12-[2-(4(S)-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-
 5 18-ene-2,3,10,16-tetraone

Silica (18g, Merck Kieselgel 60) was added to a solution of the product of step (a) (300mg) in dichloromethane (100ml). Volatiles were then removed in vacuo at room temperature and the resulting freely flowing
 10 powder was stored at 8°C for 16 hours. The support was then washed with acetone containing triethylamine and the solvent was evaporated in vacuo to an oil. Chromatography on silica eluting with hexane in an acetone gradient then gave the title compound as a foam (79mg).

15 MS (FAB): 772.83 [M+H-H₂O]⁺; 812.85 [M+Na]⁺; 874.65 [M+Rb]⁺

¹³C NMR (CDCl₃) δ: (Major rotamer) 212.2 (C16); 196.2 (C2); 169.2 (C10); 165.1 (C3); 138.0 (C19); 131.3 (C29); 130.2 (C31); 124.1 (C18); 97.2 (C1); 75.3 (C23); 69
 20 (C35); 56.1 (C9); 53.4 (C17); 49.1 (C20); 37.7 (C5); 34.9 (C13); 34.5 (C27); 30.5 (C32); 26.3 (C21); 20.8 (C7); 20.3 (C41).

c) 1-Hydroxy-12-[2-(cyclohex-3-enyl)-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-
 25 azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The title compound was prepared from the product of step (a) following the method of Example 49(a)-(c). Example 55
1,14-Dihydroxy-12-(2-cyclohexyl-1-methylvinyl)-23,25-

dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 49 (60mg) in dry methanol (12ml) was added 10% Pd-on-C (100mg) and the resulting suspension was stirred in an ice bath for one hour under an atmosphere of hydrogen. The reaction mixture was then filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the title compound as a foam (44mg).

MS (plasma spray): 724.56 [M+H-2H₂O]⁺; 742.54 [M+H-H₂O]⁺; 760.63 [M+H]⁺; 777.61 [M+NH₄]⁺

MS (FAB): 844.86 [M+Rb]⁺

¹³C NMR (CDCl₃) δ: (Major rotamer) 213.1 (C16); 195.9 (C2); 168.7 (C10); 164.4 (C3); 138.0 (C19); 131.9 (C31); 130.3 (C29); 123 (C18); 96.7 (C1); 74.9 (C23); 73.3 (C25); 72.5 (C24); 69.8 (C14); 56.3 (C9); 52.6 (C17); 48.3 (C20); 43.1 (C15); 39.3 (C13); 38.8 (C5); 36.2 (C32); 34.2 (C27); 20.1 (C43); 9.2 (C38).

Example 56

1,14-Dihydroxy-12-[2-cyclohexyl-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 50 (15mg) in dry methanol (4ml) was added 10% Pd-on-C (6mg) and the resulting suspension was stirred in an ice bath for one hour under an atmosphere of hydrogen. The reaction mixture was then filtered and concentrated to an oil in vacuo.

Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the title compound as a foam (14mg).

MS (FAB): 831 [M+Rb]⁺

5 Example 57

1-Hydroxy-12-[2-cyclohexyl-1-methylvinyl]-23,25-dimethoxy-17-ethyl-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

The title compound was prepared from the product of Example 53 following the method of Example 55.

Example 58

1-Hydroxy-12-[2-cyclohexyl-1-methylvinyl]-23,25-dimethoxy-17-propyl-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

15 The title compound was prepared from the product of Example 54 following the method of Example 55.

Example 59

17-Allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

a) 17-Allyl-1-hydroxy-12-[2-(4-^tbutyldimethylsilyloxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a cold (0°C) stirred solution of FR-900506 (1g) in dry dichloromethane (25ml) containing 2,6-dimethylpyridine

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(5ml) under nitrogen was added ^tbutyldimethylsilyltriflate (2ml) until all the starting material had disappeared. The reaction mixture was then quenched with water and, after stirring for 5 minutes at room temperature, was extracted with diethyl ether. The ether extracts after washing with dilute aqueous hydrochloric acid (1N) (x2), saturated aqueous sodium hydrogen carbonate solution and brine were dried (MgSO₄), filtered and concentrated in vacuo to give the subtitle compound as an oil (1.28g).

b) 17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product from step (a) in methanol (100ml) containing pyridinium p-toluene sulphonate was stirred for 18 hours at room temperature. Volatiles were then removed in vacuo and the residue was dissolved in diethyl ether. The ethereal solution after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate solution and brine was dried (MgSO₄), filtered and evaporated in vacuo to give the subtitle compound as a pale yellow foam (0.97g).

c) 17-Allyl-1-hydroxy-12-[2-(4-(imidazol-1-yl(thiocarbonyl)oxy)-3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-

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tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of step (b) (280mg) in dry distilled dichloroethane (40ml) containing 1,1'-thiocarbonyldiimidazole (2g) was heated under reflux for 36 hours under an atmosphere of nitrogen. Volatiles were then removed in vacuo and the residue was chromatographed on silica eluting with dichloromethane/acetone [9:1] to give the subtitle compound (105mg) as a foam.

d) 17-Allyl-1,2-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione

A solution of the product of step (c) (105mg) in dry benzene (25ml) containing AIBN (2,2'-bisisobutyronitrile) (3mg) was heated to 40°C under nitrogen. Tributyltin hydride (0.1ml) was then added dropwise by syringe. The temperature was then raised to 60°C over 5 minutes and a further 0.1 ml of tributyltin hydride was added. The temperature was then further raised to 90°C over 10 minutes and an additional 0.1ml of tributyltin hydride was added. After a further 10 minutes no starting material remained and volatiles were removed in vacuo after cooling to room temperature. Chromatography on silica then gave the subtitle compound as an oil (85mg).

e) 17-Allyl-1-hydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-14-^tbutyldimethylsilyloxy-23,25-dimethoxy-

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13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of the product of step (d) (85mg) in glacial acetic acid (10ml) containing copper (II) acetate (1g) was
 5 heated at 80°C for 5 minutes. The cooled reaction mixture was then poured into saturated aqueous sodium hydrogen carbonate solution and this was extracted with diethyl ether. The ether extracts were then dried (MgSO₄), filtered and concentrated to an oil in vacuo.
 10 Chromatography on silica eluting with acetone/hexane [2:5] then gave the subtitle compound as a foam (40mg).

f) 17-Allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-
dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
 15 tetraone

To a solution of the product of step (e) (40mg) in acetonitrile (8ml) was added 40% aqueous hydrofluoric acid (1ml). After stirring for 1 hour at room temperature the reaction mixture was poured into saturated aqueous sodium
 20 hydrogen carbonate solution and the mixture was extracted with diethyl ether. The ether extracts were then dried (MgSO₄), filtered and concentrated to an oil in vacuo.
 Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the subtitle compound
 25 as a foam (20mg).

MS (plasma spray): 752.73 [M+H-2H₂O]⁺; 770.76

[M+H-H₂O]⁺; 788.77 [M+H]⁺; 805.79 [M+NH₄]⁺

¹³C NMR (CDCl₃) δ: (Major rotamer) 212.9 (C16);

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196.2 (C2); 169 (C10); 164.7 (C3); 139.0 (C19); 135.6 (C41); 131.6 (C29); 130.5 (C31); 122.4 (C18); 116.7 (C42); 97 (C1); 78.9 (C34); 77 (C12); 75.2 (C23); 73.7 (C25); 72.8 (C24); 70.1 (C14); 56.4 (C9); 52.7 (C17); 48.5 (C20); 43.1 (C15); 39.7 (C13); 39.2 (C5); 26.3 (C21); 21.2 (C7); 20.5 (C44); 14.1 (C30); 9.4 (C39).

g) 17-Allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-
10 trione

Hydrogen sulphide gas was bubbled through a solution of the product of step (f) (40mg) in pyridine (2ml) and dimethylformamide (0.1ml) for 2 hours at room temperature. After standing for 4 hours at room temperature dilute
15 aqueous hydrochloric acid was added and the reaction mixture was extracted with ethyl acetate. The ethyl acetate extract was then dried (MgSO₄), filtered and concentrated in vacuo. Chromatography on silica eluting with ethyl acetate then gave the title compound as a foam
20 (25mg).

MS (FAB): 858 (M+Rb)⁺; 796 (M+Na)⁺; 774 (M+H)⁺;
756 (M-OH)⁺

¹³C NMR (CDCl₃) δ: 214.3 (C16); 174 (C3); 169.4 (C10); 141.2 (C19); 135.4 (C41); 131.6 (C29); 129.8 (C31);
25 121.4 (C18); 116.6 (C42); 97.8 (C1); 78.9 (C34); 48.4 (C20); 20.7 (C7); 14.3 (C30); 9.7 (C39)

Example 60

17-Allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-

methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-
dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
tetraone

and

5 17-Allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-
methylvinyl]-23,25-dimethoxy-1,13,19,21,27-pentamethyl-
11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

a) 17-Allyl-1-chloro-14-hydroxy-12-[2-(4-hydroxy-3-
10 methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

A solution of FR-900506 (500mg) in dry dichloromethane
(25ml) was added dropwise over 1 minute to a stirred, cool
15 (0°C) solution of thionyl chloride (0.45ml) and pyridine
(1.11ml) in dry dichloromethane (20ml) under nitrogen.
After 20 minutes, saturated aqueous sodium hydrogen
carbonate solution was added and the mixture was stirred at
room temperature for 20 minutes. The organic extract was
20 then separated and washed with dilute aqueous hydrochloric
acid (1M, 20ml), water (20ml) and brine (10ml) before being
dried (MgSO₄), filtered and evaporated in vacuo to give
the sub-title compound as an an oil (512mg).

b) 17-Allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclo
25 hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-
tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]octacos-
18-ene-2,3,10,16-tetraone

and

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17-Allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-1,13,19,21,27-pentamethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

5 To a cold (-50°C), stirred suspension of copper (I) iodide (463mg) in dry diethyl ether (20ml) under nitrogen was added a dilute (1.1M) solution of methyl lithium in ether (4.42ml). After stirring for 30 minutes at -40°C the reaction mixture was cooled to -70°C and a solution of the product from step (a) (400mg) in dry ether (20ml) was added dropwise. After stirring for 20 minutes, saturated aqueous ammonium chloride solution was added and the reaction mixture was allowed to warm to room temperature. The ethereal layer was then separated and was washed with water (20ml) and brine (20ml) before being dried (MgSO₄), filtered and evaporated to an oil in vacuo. Chromatography on silica then gave a first isomer of the first title compound (Isomer A, 5mg), a second isomer of the first title compound (Isomer B, 20mg), and the second title compound (4.5mg).

MS (FAB):

Isomer A - 770.8 [M+H-H₂O]⁺; 788.8 [M+H]⁺; 810.8 [M+Na]⁺; 872.6 [M+Rb]⁺

Isomer B - 872.4 [M+Rb]⁺

25 2nd title compound - 784.8 [M+H-H₂O]⁺; 802.8 [M+H]⁺; 824.8 [M+Na]⁺; 886.5 [M+Rb]⁺

¹³C NMR (CHCl₃) δ:

Isomer A - 211.4 (C16); 200.7 (C2); 169 (C10); 165.6

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(C3); 139.6 (C19); 135.7 (C41); 131.9 (C31); 131.2 (C29);
 122.4 (C18); 116.5 (C42); 84.2 (C34); 80.5 (C12); 78.3
 (C1); 76.9 (C23); 75.2 (C24); 74.9 (C25); 73.5 (C35); 68.5
 (C14); 53.4 (C17); 52 (C9); 47.7 (C20); 45.5 (C15); 44.3
 5 (C5); 40.1 (C13); 35.2 (C40); 34.9 (C32); 34.8 (C22); 34.6
 (C33); 32.7 (C26); 31.5 (C27); 31.2 (C36); 30.5 (C37); 27.1
 (C21); 25.8 (C8); 24.9 (C6); 20.8 (C7); 20.5 (C44); 17.1
 (C43); 16.4 (C47); 13.3 (C30); 10.1 (C39)

Isomer B - 213.2 (C16); 197 (C2); 170.2 (C10); 163.8
 10 (C3); 137.3 (C19); 135.2 (C41); 131.9 (C29); 128.5 (C31);
 123.4 (C18); 116.7 (C42); 84.1 (C34); 83.5 (C1); 79.3
 (C12); 70.2 (C14); 55.9 (C9); 51.9 (C17); 49.4 (C20); 44.7
 (C15); 40 (C5); 40.1 (C13); 38.5 (C40); 10.1 (C39)

2nd title compound - 212.4 (C16); 203.3 (C2); 169.4
 15 (C10); 167 (C3); 139.1 (C19); 135.6 (C41); 131.8 (C29);
 129.7 (C31); 123 (C18); 116.6 (C42); 84.2 (C34); 82.9 (C1);
 77.3 (C12); 69.7 (C14); 52.5 (C17); 52 (C9); 47.7 (C20);
 45.2 (C15); 44 (C5); 39.9 (C13); 14.1 (C48); 10 (C39).

20 Isomers A and B differ in their stereochemistry at C1.

Example 61

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol(methyl
 ether))-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
 25 ene-2,3,10,16-tetraone

To a solution of the compound of Example 10(a) (73mg) in
 diethyl ether (2ml) containing boron trifluoride diethyl
 etherate (0.1ml) was added an ethereal solution of

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diazomethane. After standing for 30 minutes at room temperature volatiles were removed in vacuo and the residue was chromatographed on silica eluting with hexane/acetone [4:1] to give 17-allyl-1-hydroxy-12-[2-(cyclopentyl-
 5 3-methanol(methylether))-1-methylvinyl]-23,25-dimethoxy-14-
 tbutyldimethylsilyloxy-13,19,21,27-tetramethyl-11,28-dioxa
 -4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-
 tetraone (20mg) as a foam. This was dissolved in
 acetonitrile (5ml) and 40% aqueous hydrofluoric acid
 10 (0.5ml) was then added. After stirring for 75 minutes at
 room temperature the reaction mixture was poured into ethyl
 acetate and was washed with saturated aqueous sodium
 hydrogen carbonate solution and brine before being dried,
 (MgSO₄), filtered and evaporated to an oil in vacuo.
 15 Chromatography on silica eluting with acetone/hexane [1:3]
 then gave the title compound (10mg) as a foam.
¹³C NMR (CDCl₃) δ: (Major rotamer) 213.8 (C16); 196.2
 (C2); 168.9 (C10); 164.9 (C3); 138.9 (C19); 135.6 (C40);
 122.5 (C18); 116.6 (C41); 97 (C1); 77.4 (C12); 75.2 (C23);
 20 70.1 (C14); 58.8 (cyclopentylCH₂OCH₃); 56.3 (C9); 52.8
 (C17); 48.6 (C20); 29.7 (C8); 26.3 (C21); 24.6 (C6); 21.1
 (C7); 20.4 (C43); 14.1 (C30); 9.5 (C38)
 MS (FAB): 872 [M+Rb]⁺; 810 [M+Na]⁺; 788 [M+H]⁺.

Example 62

25 17-Allyl-1,14-dihydroxy-12-[2-(4-amino-3-methoxycyclohexyl)-
1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-
11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-
2,3,10,16-tetraone

a) 17-Allyl-1-hydroxy-12-[2-(4-azido-3-methoxycyclohexyl)-
1-methylvinyl]-23,25-dimethoxy-14-^tbutyldimethylsilyloxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

5 To a stirred, cold (-20°C) solution of the product of
Example 39(b) (0.19g) in dry distilled dichloromethane
(7ml) containing dry pyridine (0.63ml) under nitrogen was
added trifluoromethanesulphonic anhydride (0.41ml). After
20 minutes at -20°C saturated aqueous sodium hydrogen
10 carbonate solution (3ml) was added and the reaction mixture
was extracted with diethyl ether. The organic extracts
were then washed with saturated aqueous sodium hydrogen
carbonate solution, dilute aqueous hydrochloric acid (1N),
and saturated aqueous sodium hydrogen carbonate solution
15 before being dried (MgSO₄), filtered and concentrated to
an oil in vacuo. This material was dissolved in dry DMF
(5ml) and sodium azide (0.5g) was added. After stirring
for 30 minutes at room temperature the reaction mixture was
poured into water and this was then extracted with ethyl
20 acetate. The organic extract after washing with brine was
dried (MgSO₄), filtered and concentrated to an oil in
vacuo. Chromatography on silica then gave the subtitle
compound (83mg) as a foam.

b) 17-Allyl-1-hydroxy-12-[2-(4-amino-3-methoxycyclohexyl)-
25 1-methylvinyl]-23,25-dimethoxy-14-^tbutyldimethylsilyloxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

T a stirred solution of the product of step (b) (50mg) in

dry, distilled methanol (5ml) under nitrogen was added 1,3-propanedithiol (0.03ml) and triethylamine (0.04ml). After stirring for 1 hour at room temperature the reaction mixture was columned on silica eluting with hexane/acetone [3:1] to give the subtitle compound as a foam (37mg).

^{13}C NMR (CDCl_3) δ : (Major rotamer) 209.6 (C16); 196.5 (C2); 169.1 (C10); 164.7 (C3); 138.5 (C19); 135.7 (C41); 133.3 (C29); 128.3 (C31); 123.2 (C18); 116.6 (C42); 97.6 (C1); 82.4 (C34); 56.4 (C9); 53.7 (C17); 49.3 (C20); 43.7 (C15); 40.6 (C13); 39.2 (C5); 10.5 (C39).

MS (FAB): 1001.6 $[\text{M}+\text{Rb}]^+$

c) 17-Allyl-1,14-dihydroxy-12-[2-(4-amino-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of step (b) (35 mg) in acetonitrile (7ml) was added 40% aqueous hydrofluoric acid (0.5ml). After stirring for 2.5 hours at room temperature the reaction mixture was poured into ethyl acetate and the separated organic extract was then washed with saturated aqueous sodium hydrogen carbonate solution and brine before being dried (MgSO_4), filtered and evaporated to an oil in vacuo. Column chromatography on silica eluting with hexane/acetone [2:1] then gave the title compound (15mg) as a foam.

^{13}C NMR (CDCl_3) δ : (Major rotamer) 212.9 (C16); 196.2 (C2); 169.1 (C10); 164.8 (C3); 139.1 (C19); 135.7 (C41); 132.9 (C29); 128.5 (C31); 122.6 (C18); 116.8 (C42); 97.2

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(C1); 82.9 (C34); 78 (C12); 75.4 (C23); 73.8 (C25); 73.0
(C2 4); 70.2 (C14); 57.1 (C9); 53.1 (C17); 48.7 (C20); 43.3
(C15); 39.8 (C13); 39.4 (C5); 24.1 (C6); 21.3 (C7); 20.6
(C44); 14.2 (C30); 9.7 (C39).

5 MS (FAB): 888.5 [M+Rb]⁺; 826.7 [M+Na]⁺; 786.7
[M+H-H₂O]⁺

Example 63

17-Allyl-1,14-dihydroxy-12-[2-(4-acetamido-3-methoxycyclo
hexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
10 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
ene-2,3,10,16-tetraone

~~a) 17-Allyl-1-hydroxy-12-[2-(4-acetamido-3-methoxycyclo
hexyl)-1-methylvinyl]-23,25-dimethoxy-14-^tbutyldimethyl
silyloxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo~~
15 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 62(b) (20mg) in dry
dichloromethane (3ml) was added pyridine (0.1ml) and acetyl
chloride (0.1ml). After stirring for 10 minutes at room
temperature the reaction mixture was poured into water and
20 this was then extracted with diethyl ether. The organic
~~extract was then washed with dilute aqueous hydrochloric
acid and brine before being dried (MgSO₄), filtered and
evaporated to an oil in vacuo. Chromatography on silica
eluting with hexane/acetone [3:1] then gave the subtitle~~
25 compound (15mg) as an oil.

b) 17-Allyl-1,14-dihydroxy-12-[2-(4-acetamido-3-methoxy
cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-

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ene-2,3,10,16-tetraone

A portion of the product from step (a) (13mg) was dissolved in acetonitrile (4ml) and to this was added 40% aqueous hydrofluoric acid (0.1ml). After stirring for 2 hours at room temperature the reaction mixture was poured into ethyl acetate and the separated organic extract was then washed with water, saturated aqueous sodium hydrogen carbonate solution and brine before being dried (MgSO_4), filtered and evaporated to an oil in vacuo. Column chromatography on silica eluting with hexane/acetone [2:1] then gave the title compound (8mg) as a foam.

^{13}C NMR (CDCl_3) δ : (Major rotamer) 212.4 (C16); 196.2 (C2); 169 (C10); 164.7 (C3); 139 (C19); 135.5 (C41); 122.4 (C18); 116.7 (C42); 97 (C1); 9.4 (C39)

^1H NMR (CDCl_3) δ : 2.01 [3H, s, NHCOCH_3]

MS (FAB): 930.5 [$\text{M}+\text{Rb}$] $^+$; 868.9 [$\text{M}+\text{Na}$] $^+$

Example 64

17-Allyl-1,14-dihydroxy-12-[2-(4-formyloxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the compound of Example 39(c) (1.103g) in dry DMF (20ml) was added sodium azide (2.58g). After stirring for 2 hours at room temperature the reaction mixture was poured into water and this was then extracted with ethyl acetate. The organic extract after washing with brine was dried (MgSO_4), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with

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hexane/acetone [3:1] then gave 17-allyl-1-hydroxy-12-[2-(4-formyloxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-14-^tbutyldimethylsilyloxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (115mg) as a foam. A portion of this (71mg) was dissolved in acetonitrile (14ml) and to this was added 40% aqueous hydrofluoric acid (0.5ml). After stirring for 3.5 hours at room temperature the reaction mixture was poured into ethyl acetate and the separated organic extract was then washed with water, saturated aqueous sodium hydrogen carbonate solution and brine before being dried (MgSO₄), filtered and evaporated in vacuo to an oil. Column chromatography on silica eluting with hexane/acetone [2:1] then gave the title compound (19mg) as a foam.

¹³C NMR (CDCl₃) δ: (Major rotamer) 212.7 (C16); 196.2 (C2); 169.2 (C10); 164.8 (C3); 160.6 (OCHO-); 138.9 (C19); 135.5 (C41); 132.4 (C29); 129.5 (C31); 122.4 (C18); 116.6 (C42); 96.9 (C1); 78.7 (C34); 77.3 (C12); 75.1 (C23); 72.8 (C24); 70 (C14); 56.6 (C9); 52.7 (C17); 48.5 (C20); 43 (C15); 39.6 (C13); 39.2 (C5); 28.2 (C8); 26.2 (C21); 24.5 (C6); 21.1 (C7); 20.4 (C44); 14.1 (C30); 9.3 (C39).

MS (FAB): 916.2 [M+Rb]⁺; 854.5 [M+Na]⁺; 832.6 [M+H]⁺; 814.6 [M+H-H₂O]⁺

25 Example 65

17-Allyl-1,14-dihydroxy-12-[2-(3-oxo-cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-

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tetraone

and

17-Allyl-1,14-dihydroxy-12-[2-(3-methoxy-cyclohex-4-enyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

Silica (220g, Merck Kieselgel 60, Art. 15111) was added to a solution of the compound of Example 39(c) (250ml). Volatiles were then removed in vacuo at room temperature and the resulting freely flowing powder was stored at 8°C for 16 hours. The support was then washed with ethyl acetate and 10% acetone in ethyl acetate containing 2,6-dimethylpyridine. The combined organic extracts after washing with saturated aqueous sodium hydrogen carbonate solution, dilute aqueous hydrochloric acid (1N), saturated aqueous sodium hydrogen carbonate solution and brine were dried, (MgSO₄), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane in an acetone gradient then gave the compound of Example 39(d) (1.12g) as a foam. Further elution then gave the compound of Example 8(c) (0.5g) as a foam.

Mixed fractions were then combined, treated with 40% aqueous hydrofluoric acid as above, and re-chromatographed on silica eluting with ethyl acetate to give the first title compound (200mg).

¹³C NMR (CDCl₃) δ: (Major rotamer) 212.5 (C16); 210.7 (C34); 196.2 (C2); 169 (C10); 164.7 (C3); 139 (C19); 135.5

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(C41); 133.1 (C29); 129 (C31); 122.5 (C18); 116.7 (C42);
97.1 (C1); 77.6 (C12); 75.2 (C23); 73.7 (C25); 72.8 (C 24);
69.9 (C14); 56.3 (C9); 52.9 (C17); 48.6 (C20); 47.6 (C33);
43.5 (C15); 41.2 (C35); 39.7 (C13); 37.9 (C32); 26.2 (C21);
5 25.8 (C8); 24.5 (C6); 21.1 (C7); 20.4 (C44); 13.8 (C30);
9.7 (C39).

MS (FAB): 856 [M+Rb]⁺; 794 [M+Na]⁺; 736 [M+H-2H₂O]⁺

Further elution then gave the second title compound.

13 NMR (CDCl₃): δ (Major rotamer) 212.6 (C16); 196.2
10 (C2); 168.9 (C10); 164.6 (C3); 139.7 (C19); 135.5 (C41);
132.3 (C29); 129.9 (C31); 122.3 (C18); 116.5 (C42); 128.5
— (C35); 128.1 (C36); 96.9 (C1); 73.5 (C25); 72.7 (C24); 70.5
(C14); 56.5 (C9); 52.7 (C17); 48.4 (C20); 27.6 (C8); 26.1
— (C21); 24.4 (C6); 21 (C7); 20.3 (C44); 14 (C30); 9.3 (C39).

15 MS (FAB): 870 [M+Rb]⁺; 808 [M+Na]⁺

Example 66

17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic
acid morpholine amide)-1-methylvinyl]-23,25-dimethoxy
-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
20 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone

To a solution of the product of Example 13 was added
morpholine (0.03ml) followed by triethylamine (0.03ml) and
2-chloro-1-methylpyridinium tosylate (70mg). After
stirring for 1 hour at room temperature a further portion
25 of the tosylate (40mg) was added and stirring was continued
for 5.5 hours at room temperature. Additional
triethylamine (0.03ml) and morpholine (0.03ml) was then
add d and the reaction mixture was stirred overnight at

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room temperature. The reaction was then quenched with dilute aqueous hydrochloric acid (2M, 10ml) and the mixture was extracted with ethyl acetate. The organic extracts were then washed with saturated aqueous sodium hydrogen carbonate solution and brine before being dried (MgSO_4), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with hexane in an increasing acetone gradient then gave the title compound (30mg) as a foam.

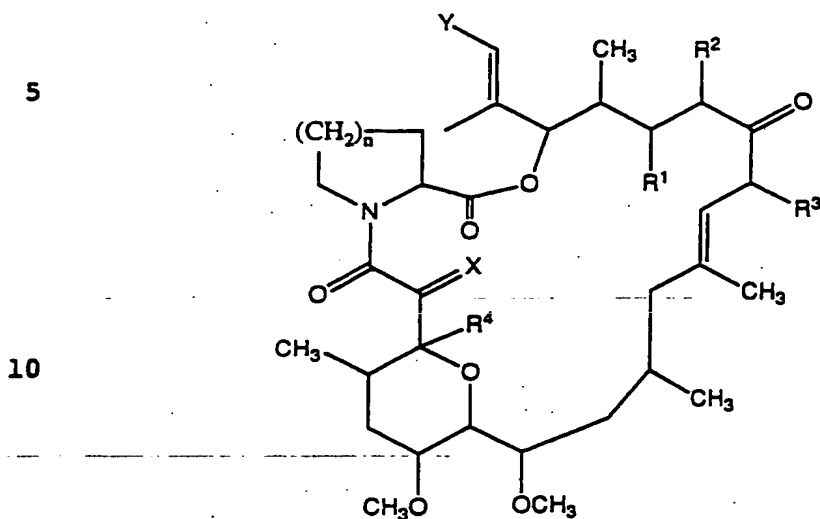
MS (FAB): 941.4 $[\text{M}+\text{Rb}]^+$; 880.2 $[\text{M}+\text{Na}]^+$; 858.4 $[\text{M}+\text{H}]^+$;
10 840.4 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$.
 ^{13}C NMR (CDCl_3) δ : (Major rotamer) 212.4 (C16); 196.2 (C2); 174.6 (cyclopentylCO); 169.1 (C10); 164.7 (C3); 138.9 (C19); 135.6 (C40); 132.5 (C29); 131.3 (C31); 122.7 (C18); 116.7 (C41); 97.1 (C1); 70.0 (C14); 67 and 66.8 (morpholine
15 CH_2O); 56.3 (C9); 52.9 (C17); 48.8 (C20); 46.1 and 42.3 (morpholine CH_2N); 27.8 (C8); 26.2 (C21); 24.5 (C6); 21.0 (C7); 20.3 (C43); 14.1 (C30); 9.9 (C38)

20

25

CLAIMS:

1. A compound of formula I,



wherein

15 R^1 represents H, OH or alkoxy;

R^2 represents H;

in addition, R^1 and R^2 may together represent a second bond between the carbon atoms to which they are attached;

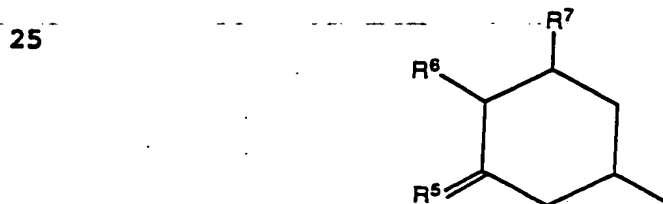
R^3 represents methyl, ethyl, propyl or allyl;

20 R^4 represents H, OH, alkyl, alkoxy, halogen, amino, S-alkyl, NRCHO or NHCO-alkyl;

n represents 1 or 2;

X represents O, (H,OH), (H,H) or =NH; and

Y represents a cyclic group of formula II,

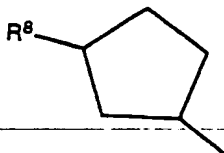


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in which R^5 represents (H,H), (H,OH), (H,methoxy) or O;
 R^6 represents H, (R)-OH, (S)-OH, alkoxy, amino,
 alkylamino, alkanoylamino, formyloxy or halogen; R^7
 represents H; and in addition R^5 and R^6 may together
 5 represent a second bond between the carbon atoms to which
 they are attached; or R^6 and R^7 may together represent
 a second bond between the carbon atoms to which they are
 attached;

or a cyclic group of formula III,

10



III

15 in which R^8 represents alkyl substituted by one or more
 groups selected from OH, alkoxy, =O, and CO_2H ; or alkenyl
 optionally substituted by one or more groups selected from
 OH, =O, or CO_2H ;

provided that

20 a) when n represents 1; R^1 represents OH; R^3
 represents allyl; R^4 represents OH; R^5 represents
 (H,methoxy); and R^6 represents (R)-OH; then X does not
 represent O;

b) when n represents 2;

25 i) R^1 represents OH; R^3 represents methyl, ethyl,
 allyl or propyl; R^4 represents OH; R^5 represents
 (H,methoxy); and R^6 represents (R)-OH; then X does not
 represent O;

- ii) when R^1 and R^2 together represent a second bond between the carbon atoms to which they are attached or each represent H; R^3 represents allyl or propyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent O;
- iii) when R^1 represents OH, methoxy or together with R^2 it represents a second bond between the carbon atoms to which they are attached; R^3 represents allyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents methoxy; then X does not represent O;
- iv) when R^1 represents H or OH; R^3 represents allyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent (H, OH);
- v) when R^1 represents H; R^3 represents propyl; R^4 represents OH; R^5 represents (H, OH); and R^6 represents (R)-OH; then X does not represent O;
- vi) when R^1 represents OH; R^3 represents ethyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent (H, OH);
- ~~vii) when R^1 and R^2 together represent a second bond between the carbon atoms to which they are attached or each represent H; R^3 represents ethyl; R^4 represents OH; R^5 represents (H, methoxy); and R^6 represents (R)-OH; then X does not represent O;~~
- viii) when R^1 represents OH; R^3 represents allyl; R^4 represents OH; R^5 represents (H, OH) or (H, methoxy); and R^6 represents (R)-OH; then X does not represent (H, H);
- ix) when R^1 represents OH; R^3 represents ethyl; R^4

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represents OH; R⁵ represents (H, methoxy); and R⁶ represents (R)-OH; then X does not represent (H, H);
 x) when R¹ represents OH; R³ represents methyl, ethyl or allyl; R⁴ represents OH; R⁵ represents (H, OH); and
 5 R⁶ represents (R)-OH; then X does not represent O; and
 xi) when R¹ represents OH; R³ represents allyl; R⁴ represents OH; R⁵ represents O; and R⁶ represents (R)-OH; then X does not represent O;

and pharmaceutically acceptable derivatives thereof.

10 2. A compound of formula I, as claimed in claim 1, wherein R¹ represents H or OH.

3. A compound of formula I, as claimed in claim 1 or claim 2, wherein R⁴ represents H, OH, alkyl, halogen or amino.

15 4. A compound of formula I, as claimed in any one of the preceding claims, wherein R⁵ represents (H, OH) or (H, methoxy).

5. A compound of formula I, as claimed in any one of the preceding claims, wherein R⁶ represents H, (R)-OH or
 20 amino.

6. A compound of formula I, as claimed in any one of the preceding claims, wherein R⁸ represents an amide of a CO₂H group or alkyl substituted by alkoxy.

7. A compound of formula I, as claimed in claim 1, which
 25 is:

17-allyl-1,14-dihydroxy-12-[2-(3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-di xa-4-azatricycl [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-

- tetraone;
- 17-allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-carboxylic acid morpholine amide)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo
- 5 [22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 17-allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 10 17-allyl-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-1,13,19,21,27-pentamethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 17-allyl-1-amino-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra
- 15 methyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 17-allyl-1-fluoro-14-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-
- 20 tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone;
- 17-Allyl-1,14-dihydroxy-12-[2-(cyclopentyl-3-methanol (methyl ether))-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-
- 25 ene-2,3,10,16-tetraone; or
- 17-Allyl-1,14-dihydroxy-12-[2-(4-amino-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-

2,3,10,16-tetra ne.

8. The use of a compound of formula I, as defined in claim 1, as a pharmaceutical.

9. A pharmaceutical composition comprising a compound of
5 formula I, as defined in claim 1, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

10. The use of a compound of formula I, as defined in claim 1, in the manufacture of a medicament for use as an immunosuppressive agent.

10 11. A method of effecting immunosuppression which comprises administering a therapeutically effective amount of a compound of formula I, as defined in claim 1, to a patient.

12. A process for the production of a compound of formula
15 I as defined in claim 1, which comprises:

(a) producing a compound of formula I in which R^1 and R^2 together represent a second carbon-carbon bond between the carbon atoms to which they are attached, by dehydration of a corresponding compound in which R^1 represents OH and
20 R^2 represents H;

(b) producing a compound of formula I in which R^1 and R^2 each represent hydrogen, by reduction of a corresponding compound in which R^1 and R^2 together represent a second carbon-carbon bond between the carbon
25 atoms to which they are attached;

(c) producing a compound of formula I in which X represents (H,OH), by reduction of a corresponding compound in which X represents O;

- (d) producing a compound of formula I in which X represents (H,H), by reduction of a corresponding compound in which X represents O;
- (e) producing a compound of formula I in which X represents O, by oxidation of a corresponding compound in which X represents (H,OH);
- (f) producing a compound of formula I in which R^4 represents alkoxy, by reaction of a corresponding compound in which R^4 represents OH and X represents (H,OH) with an alkanol;
- (g) producing a compound of formula I in which R^4 represents halogen, by reaction of a corresponding compound in which R^4 represents OH with a suitable halogenating agent;
- (h) producing a compound of formula I in which R^4 represents H or alkyl, by reaction of a corresponding compound in which R^4 represents halogen with an organometallic reagent;
- (i) producing a compound of formula I in which R^4 represents amino, by reaction of a corresponding compound in which R^4 represents halogen with ammonia;
- (j) producing a compound of formula I in which X represents =NH, by reaction of a corresponding compound in which R^4 represents halogen with ammonia;
- (k) producing a compound of formula I in which R^4 represents S-alkyl, by reaction of a corresponding compound in which R^4 represents halogen with an alkylthiol;
- (l) producing a compound of formula I in which R^4

represents NHCHO , by reaction of a corresponding compound in which R^4 represents amino with formic acid;

(m) producing a compound of formula I in which R^4 represents NHCO-alkyl , by reaction of a corresponding
5 compound in which R^4 represents amino with an alkanolic anhydride;

(n) producing a compound of formula I in which R^6 represents $(\text{S})\text{-OH}$, by elimination of a leaving group from a
corresponding compound in which R^6 represents the leaving
10 group;

(o) producing a compound of formula I in which R^6 represents H and R^5 represents O , by elimination of a
leaving group from a corresponding compound in which R^6 represents the leaving group;

15 (p) producing a compound of formula I in which R^6 and R^7 together represent a second bond between the carbon atoms to which they are attached, by elimination of a leaving group from a corresponding compound in which R^6 represents the leaving group;

20 (q) producing a compound of formula I in which Y represents a cyclic group of formula III and R^8 represents -CHO , by elimination of a leaving group from a corresponding compound in which R^6 represents the leaving group;

25 (r) producing a compound of formula I in which R^6 represents halogen, by reaction of a corresponding compound in which R^6 represents a leaving group with halide ion;

(s) producing a compound of formula I in which R^5 and

R^6 together represent a second bond between the carbon atoms to which they are attached, by elimination of halogen and alkoxy from a corresponding compound in which R^5 represents alkoxy and R^6 represents halogen;

5 (t) producing a compound of formula I in which R^5 represents (H,H) and R^6 represents H, by reduction of a corresponding compound in which R^5 and R^6 together represent a second bond between the carbon atoms to which they are attached;

10 (u) producing a compound of formula I in which R^6 represents H, by the action of hydride on a corresponding compound in which R^6 represents a leaving group;

(v) producing a compound of formula I in which R^6 represents amino, by reduction of a corresponding compound
15 in which R^6 represents azido;

(w) producing a compound of formula I in which R^6 represents alkylamino or alkanoylamino, by reaction of a corresponding compound in which R^6 represents amino with a suitable alkylating or acylating reagent;

~~20 (x) producing a compound of formula I in which R^8 represents alkyl substituted by OH, by reduction of a corresponding compound in which R^8 represents alkyl substituted by =O;~~

(y) producing a compound of formula I in which R^8
25 includes a carboxylic acid group, by oxidation of a corresponding compound in which R^8 includes an aldehyde group; or

(z) producing a compound of formula I in which R^8

- 99 -

represents optically substituted alkenyl, by a Wittig reaction between a corresponding compound in which R^8 includes an aldehyde and an appropriate Wittig reagent.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00393

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC ⁵ C 07 D 498/18, C 07 H 19/01, A 61 K 31/40, 31/445, 31/70 IPC :/(C 07 D 498/18, 311:00, 273:00, 209:00)(C 07 D 498/18, 311:00, 273:00, 221:00)		
II. FIELDS SEARCHED		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
IPC ⁵	C 07 D 498/00, C 07 H 19/00, A 61 K 31/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP, A, 0184162 (FUJISAWA) 11 June 1986 see claims 1,18 cited in the application ---	1,10
X	EP, A, 0323042 (FISONS) 5 July 1989 see claim 8 cited in the application ---	10
X	EP, A, 0356399 (SANDOZ) 28 February 1990 see claims 1,8 cited in the application -----	1,10
<p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
29th May 1991	3 JUL 1991	
International Searching Authority	Signature of Authorised Officer	
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 11, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1 (iv): methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9100393
SA 45510

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 18/07/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0184162	11-06-86	AU-B- 592067	04-01-90
		AU-A- 5059685	12-06-86
		JP-A- 3072483	27-03-91
		JP-A- 3072484	27-03-91
		JP-A- 61148181	05-07-86
		US-A- 4956352	11-09-90
		US-A- 4894366	16-01-90
EP-A- 0323042	05-07-89	US-A- 4929611	29-05-90
		AU-A- 2822889	05-07-89
		EP-A- 0346427	20-12-89
		WO-A- 8905304	15-06-89
EP-A- 0356399	28-02-90	JP-T- 2502463	09-08-90
		AU-A- 4024689	01-03-90
		JP-A- 2167287	27-06-90
		US-A- 5011844	30-04-91

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82